(54) Title: ASCORBIC ACID ANALOGS FOR METALLORADIOPHARMACEUTICALS

(57) Abstract: The invention relates to the use of ascorbic acid analogs as buffering reagents and chelating agents for the preparation of metalloradiopharmaceuticals. Also, invention relates to the use of ascorbic acid as a buffering reagent, a chelating agent, and a stabilizer for the preparation and stabilization of radiopharmaceuticals and processes for making and using the same.

TITLE

ASCORBIC ACID ANALOGS FOR METALLORADIOPHARMACEUTICALS

5   FIELD OF THE INVENTION

     This invention is related to the use of ascorbic
acid analogs as buffering reagents and chelating agents
for the preparation of metalloradiopharmaceuticals.
10   This invention is particularly related to the use of
ascorbic acid as a buffering reagent, a chelating agent,
and a stabilizer for the preparation and stabilization
of radiopharmaceuticals. This invention is also related
to processes of making stable radiopharmaceutical
15   compositions using ascorbic acid analogs as buffering
agents, chelating agents, and stabilizers.

                    BACKGROUND

     Radiopharmaceuticals are drugs containing a
20   radionuclide. Radiopharmaceuticals are used routinely
in nuclear medicine for the diagnosis or therapy of
various diseases. They are typically small organic or
inorganic compounds with a definite composition. They
can also be macromolecules, such as antibodies or
25   antibody fragments, that are not stoichiometrically
labeled with a radionuclide. Radiopharmaceuticals form
the chemical basis for the diagnosis and therapy of
various diseases. The in vivo diagnostic information is
obtained by intravenous injection of the
30   radiopharmaceutical and determining its biodistribution
using a gamma camera. The biodistribution of the
radiopharmaceutical depends on the physical and chemical

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properties of the radiolabeled compound and can be used
to obtain information about the presence, progression,
and state of disease.

Radiopharmaceuticals can be divided into two
5   primary classes: those whose biodistribution is
determined exclusively by their chemical and physical
properties; and those whose ultimate distribution is
determined by their receptor binding or other biological
interactions. The latter class is often called target-
10 specific radiopharmaceuticals.

Metalloradiopharmaceuticals include a metallic
radionuclide. A target-specific
metalloradiopharmaceutical can be divided into four
parts: a targeting molecule, a linker, a bifunctional
15 Chelator (BFC), and a metallic radionuclide. The
targeting molecule serves as a vehicle, which carries
the radionuclide to the receptor site at the diseased
tissue. The targeting molecules can be macromolecules
such as antibodies or small biomolecules (BM), including
20 peptides, peptidomimetics, and non-peptides. The choice
of biomolecule depends upon the targeted disease or
disease state. The radionuclide is the radiation
source. The selection of metallic radionuclide depends
on the intended medical use (e.g., diagnostic or
25 therapeutic) of the target specific
metalloradiopharmaceutical. The BFC is covalently
attached to the targeting molecule either directly or
through a linker and binds strongly to the metallic
radionuclide via several coordination bonds. Selection
30 of a BFC is largely determined by the nature and
oxidation state of the metallic radionuclide. The
linker can be a simple hydrocarbon chain or a long
poly(ethylene glycol) (PEG) or a "naïve" poly anionic or
cationic peptide sequence, which is often used for
35 modification of pharmacokinetics. Sometimes, a

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metabolizeable linker is used to increase the blood
clearance and to reduce the background activity, thereby
improving the target-to-background ratio.

5   The use of metallic radionuclides offers many
opportunities for designing new radiopharmaceuticals by
modifying the coordination environment around the metal
with a variety of chelators. The coordination chemistry
of the metallic radionuclide will determine the geometry
of the metal chelate and the solution stability of the
10 radiopharmaceutical. Different metallic radionuclides
have different coordination chemistries, and require
BFCs with different donor atoms and chelator frameworks.
For "metal essential" radiopharmaceuticals, the
biodistribution is exclusively determined by the
15 physical properties of the metal chelate. For target-
specific radiopharmaceuticals, the "metal tag" may have
significant impact on the target uptake and
biodistribution of the radiopharmaceutical. This is
especially true for metalloradiopharmaceuticals based on
20 small molecules since in many cases the metal chelate
contributes greatly to the overall size and molecular
weight. Therefore, the design and selection of the BFC
is very important for the development of a new
diagnostic or therapeutic radiopharmaceutical.

25   Metallic radionuclides, such as ^{99m}Tc, ^{117m}Sn, ^{111}In,
^{67}Ga, ^{68}Ga, ^{89}Zr, and ^{64}Cu, have been proposed for
diagnostic imaging. Nearly 80% of radiopharmaceuticals
used in nuclear medicine are ^{99m}Tc-labeled compounds.
The reason for such a preeminent position of ^{99m}Tc in
30 clinical use is its favorable physical and nuclear
characteristics. The 6 h half-life is long enough to
allow a radiochemist to carry out radiopharmaceutical
synthesis and for nuclear medicine practitioners to
collect useful images. At the same time, it is short
35 enough to permit administration of millicurie amounts of

^{99m}Tc radioactivity without significant radiation dose to
the patient. The monochromatic 140 KeV photons are
readily collimated to give images of superior spatial
resolution. Furthermore, ^{99m}Tc is readily available from
5   commercial ^{99}Mo-^{99m}Tc generators at low cost.

For ^{99m}Tc-labeling of biomolecules, bifunctional
chelators include N_2S_2 diaminedithiols, N_2S_2
diaminedithiols, N_2S_2 monoamidemonoamidedithiols, N_3S
aminediamidethiols, N_3S triamidethiols, and HYNIC, which
10   forms various ternary ligand systems when used in
combination with tricine/water soluble phosphines, or
tricine/pyridine analogs or tricine/substituted imime-N
containing heterocycles. These ternary ligand systems
have been disclosed in U.S. Patent No. 5,744,120; U.S.
15   Patent No. 6,010,679; U.S. Patent No. 5,879,659; and PCT
Patent Application WO 98/53858. Various ^{99m}Tc-labeling
techniques have been described in several reviews (Liu,
S. and Edwards, D. S. *Chem. Rev.* **1999,** *99*, 2235-2268;
Jurisson, S. and Lydon, J. D. *Chem. Rev.* **1999,** *99*,
20   2205-2218; Liu et al. *Bioconjugate Chem.* **1997**, *8*, 621-
636). After radiolabeling, the resulting reaction
mixture may optionally be purified using one or more
chromatographic methods, such as Sep-Pack or high
performance liquid chromatography (HPLC). The preferred
25   radiolabeling procedures are those in which the
chelation can be achieved without post-labeling
purification.

Metallic radionuclides, including ^{90}Y, ^{177}Lu, ^{149}Pm,
^{153}Sm, ^{166}Ho, ^{211}At, ^{47}Sc, ^{109}Pd, ^{105}Rh, $^{186/188}$Re, and ^{67}Cu, are
30   potentially useful for radiotherapy. Among these
radionuclides, lanthanide radioisotopes are of
particular interest. There are several lanthanide
isotopes to choose, including low energy β-emitter ^{177}Lu,
medium energy β-emitters, ^{149}Pm and ^{153}Sm, and high-energy
35   β-emitters, ^{166}Ho and ^{90}Y. Yttrium and lanthanide metals

share similar coordination chemistry. The chelator
technology and their coordination chemistry are well
developed and well understood.

For radionuclides, such as ^{90}Y, ^{111}In, ^{67}Ga, ^{68}Ga,
5   ^{89}Zr, ^{62}Cu, ^{64}Cu and ^{67}Cu, diethylenetriaminepentaacetic
acid (DTPA), tetraazacyclododecane-1,4,7,10-tetracetic
acid (DOTA) and their derivatives would be the
candidates of choice as BFCs. The macrocyclic chelators
such as DOTA are known to form highly stable metal
10 chelates due to their highly preorganized macrocyclic
ligand framework. Krejcarek and Tucker (*Biochem.
Biophys. Res. Commun.* **1976**, *77*, 581-588) developed an
activated DTPA analog via a mixed anhydride, which can
be linked to proteins. Later, Hnatowich et al (*Science*
15 **1983**, *220*, 613-616) used the cyclic anhydride of DTPA
for the same purpose. These linear BFCs bond to various
metal ions and form thermodynamically stable metal
chelates. However, metal chelates of linear BFCs are
kinetically labile, which contributes to the loss of
20 radionuclide from the metal chelate and often leads to
severe bone marrow toxicity. Gansow et al (*Bioconjugate
Chem.* **1991**, *2*, 187-194; *Inorg. Chem.* **1986**, *25*, 2772-
2781) prepared a series of substituted DTPA analogs,
which form metal chelates with improved solution
25 stability.

Meares and coworkers were the first to synthesize
macrocyclic BFCs (*Anal. Biochem.* **1985**, *148*, 249-253;
Nucl. Med. Biol. **1986**, *13*, 311-318; *J. Am. Chem. Soc.*
1988, *110*, 6266-6267), which form ^{67}Cu and ^{90}Y chelates
30 with high thermodynamic stability and kinetic inertness.
Macrocyclic chelants with three-dimensional cavities are
of particular interest because of the high stability of
the metal chelates, the substantial selectivity for
certain metal ions, either by enforcing a specific
35 spatial arrangement of donor atoms or by introducing

different donor atoms into the ligand backbone, and
their capability to adopt a preorganized conformation in
the unchelated form. The higher the degree of
preorganization of an unchelated ligand, the more stable
5   the complex will be.

Rhenium has two isotopes, ^{186}Re and ^{188}Re, which
might be useful in tumor therapy. ^{186}Re has a half-life
of 3.68 days with β—emission (Emax = 1.07 MeV, 91%
abundance) and a gamma-photon (E = 137 keV, 9%
10   abundance) which should allow imaging during therapy.
^{188}Re has a half-life of 16.98 h with an intense β-
emission (Emax = 2.12 MeV, 85% abundance) and 155 keV
gamma photons (15% abundance). The related chemistry,
medical applications, and antibody labeling with $^{186/188}$Re
15   by direct and indirect methods have recently been
reviewed (Fritzberg, A. R. et al. *Pharmaceutical Res.*
1988, 5, 325-334; Griffiths, G. L. et al. *Bioconjugate
Chem.* **1992**, 3, 91-99; Dilworth, J. R. and Parrott, S.
J. *Chem. Soc. Rev.* **1998**, 27, 43-55). Since the rhenium
20   chemistry is very similar to technetium chemistry due to
the periodic relationship, the methods used for antibody
labeling with ^{99m}Tc should apply to that with $^{186/188}$Re.

Identifying the most appropriate isotope for
radiotherapy is often a difficult task and requires
25   weighing a variety of factors. These include tumor
uptake and retention, blood clearance, rate of radiation
delivery, half-life and specific activity of the
radionuclide, and the feasibility of large-scale
production of the radionuclide in an economical fashion.
30   The key point for a therapeutic radiopharmaceutical is
to deliver the requisite amount of radiation dose to the
tumor cells and to achieve a cytotoxic or tumoricidal
effect while not causing unmanageable side-effects.

The physical half-life of the therapeutic
35   radionuclide should match the biological half-life of

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the target-specific radiopharmaceutical at the tumor
site. If the half-life of the radionuclide is too
short, much of the decay will have occurred before the
radiopharmaceutical has reached maximum

5   target/background ratio. On the other hand, too long a
half-life would cause unnecessary radiation dose to
normal tissues. Ideally, the radionuclide should have a
long enough half-life to attain a minimum dose rate (>
0.4 Gy/h) and to irradiate all the cells during the most

10   radiation sensitive phases of the cell cycle. The half-
life of a radionuclide has to be long enough to allow
adequate time for manufacturing, release, and
transportation of the radiopharmaceutical.

Other practical considerations in selecting a
15   radionuclide for a given targeting biomolecule for tumor
therapy include availability and quality. The purity
has to be sufficient and reproducible, as trace amounts
of impurities can affect the radiolabeling and the
radiochemical purity of the radiopharmaceutical. The

20   target receptor sites in tumors are typically limited in
number. This requires that the chosen radionuclide have
high specific activity. The specific activity depends
primarily on the method of production and separation
technique of the radionuclide. Trace metal contaminants

25   must be minimized as they often compete with the
radionuclide for the BFC and their metal complexes
compete for receptor binding with the radiolabeled BFC-
BM conjugate.

For tumor therapy, both α and β-emitters have been
30   investigated. Alpha particles are particularly good
cytotoxic agents because they dissipate a large amount
of energy within one or two cell diameters. Most
α-emitters are heavy elements that decay to hazardous
daughter products and their penetration range is limited
35   to only 50 μm in tissue. The short-ranged particle

emitters are more attractive if the radiopharmaceutical
is internalized into tumor cells. Auger electron
emitters are shown to be very potent but only if they
can cross the cell membrane and come into close

5   proximity with the nucleus. This creates extra
challenges for the design of new therapeutic
metalloradiopharmaceuticals. The β-particle emitters
have relatively long penetration range (2 - 12 mm in the
tissue) depending upon the energy level. The long-range

10 penetration is particularly important for solid tumors
that have heterogeneous blood flow and/or receptor
expression. The β-particle emitters yield a more
homogeneous dose distribution even when they are
heterogeneously distributed within the target tissue.

15 Depending on the tumor size and location, the choice of
the β-emitter may be different. For example, medium or
low energy β-emitters such as ^{153}Sm and ^{177}Lu are better
for smaller metastases while high-energy β-emitters such
as ^{90}Y are used for larger tumors.

20   The choice of radiolabeling approach depends on the
type of biomolecules to be labeled and the purpose of
the study. Various radiolabeling techniques for
radionuclides, including ^{90}Y, ^{111}In, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{62}Cu,
^{64}Cu and ^{67}Cu, have been described in several reviews

25 (Parker, D. *Chem. Soc. Rev.* **1990**, *19*, 271-291; Liu, F.
and Wu, C. *Pure & Appl. Chem.* **1991**, *63*, 427-463;
Anderson, C. J. and Welch, M. J. *Chem. Rev.* **1999,** *99*,
2219-2234; Volkert, W. A. and Hoffman, T. J. *Chem.
Rev.* **1999,** *99*, 2269-2292; Liu, S and Edwards, D. S.

30 *Bioconjugate Chem.* **2001**, *12*, 7-34).

    There are two general approaches, the pre-labeling
approach and the post-labeling approach, useful for the
radioabeling of biomolecules with lanthanide
radionuclides. In the post-labeling approach, a BFC is

35 first attached to the biomolecule either directly or via

a linker to form the BFC-BM conjugate. The
radiolabeling can be accomplished simply by the reaction
of the BFC-BM conjugate with the radiometal chloride in
a buffer solution in the presence of weak chelating
5   agent, if necessary. DTPA-conjugated biomolecules
usually have very high radiolabeling efficiency (fast
and high yield labeling), and can be readily labeled
within 10 min at room temperature and pH 5 – 7. The
high radiolabeling efficiency can be attributed to the
10   flexibility of the linear chelator backbone of DTPA
analogs. However, the radiolabeling kinetics of DOTA-
conjugated biomolecules is usually slow. In this case,
higher pH and elevated temperatures are often needed to
achieve fast labeling and high radiolabeling yield. The
15   post-labeling approach is useful for biomolecules that
are not sensitive to the harsh radiolabeling conditions
present in the chelation step. For biomolecules, which
are sensitive to heating, the pre-labeling approach
might be the best alternative.

20   The pre-labeling approach involves formation of the
metal chelate with a BFC, and conjugation of the M-BFC
chelate to a biomolecule in a separate step on the
tracer level. In this approach, the chemistry is well
defined, and the biomolecule is not exposed to the harsh
25   conditions used in the chelation step. For research
purposes, this approach is very useful to demonstrate
the proof of principle in a short period of time.
However, this approach is too complex and time consuming
for routine clinical use. It is also not practical for
30   large-scale production, since it involves
chromatographic separations of radiolabeled molecules at
high levels of radioactivity.

During radiolabeling, the pH of the reaction
mixture is often controlled with a buffering agent to
35   assure the reproducibility for the radiochemical purity

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of the radiopharmaceutical. The choice of a buffering
agent depends upon the optimum pH value for chelation.
Ammonium acetate is often used for the ^{90}Y- or ^{111}In-
labeling of DTPA- and DOTA-conjugated biomolecules. The

5   buffer concentration is normally 0.1 - 0.5 M.

      A radiopharmaceutical composition including β-
emitting radionuclides may undergo radiolysis during the
preparation, release, transportation, and storage of the
radiopharmaceutical composition. During radiolysis,

10 emissions from the radionuclide attack other
constituents of the complex or compound, or other
compounds in proximity, which results in inter- and
intramolecular decomposition. Radiolytic decay can
result in decomposition or destruction of the radiometal

15 chelate or the biologically active targeting molecule.
Radioactivity that is not linked to the targeting
biomolecule will accumulate in non-targeting tissues.
Decomposition of the radiopharmaceutical composition
prior to or during administration dramatically decreases

20 the targeting potential and thus increases the toxicity
of the therapeutic radiopharmaceutical composition.
Thus, it is important to ensure that the radionuclide is
linked to the targeting moiety and to ensure that
specificity of the targeting agent is preserved.

25   Radiolysis is caused by the formation of free
radicals such as hydroxyl and superoxide radicals
(Garrison, W. M. *Chem. Rev.* **1987**, *87*, 381-398). Free
radicals are very reactive towards organic molecules.
The reactivity of these free radical towards organic

30 molecules is a major factor influencing the solution
stability of a therapeutic radiopharmaceutical
composition. Stabilization of the therapeutic
radiopharmaceutical composition is a recurrent challenge
in the development of target-specific therapeutic

35 radiopharmaceuticals. Therefore, it is very important to

use a radical scavenger as a stabilizer to minimize radiolysis of the radiolabeled biomolecules.

A stabilizer is a "radical scavenging antioxidant" that readily reacts with hydroxyl and superoxide
5   radicals. The stabilizing agent for therapeutic radiopharmaceutical composition should possess the following characteristics: low or no toxicity when it is used for human administration, no interference with the delivery or receptor binding of the radiolabeled
10   compound to the target cells or tissue(s), and the ability to stabilize the therapeutic radiopharmaceutical for a reasonable period of time (e.g., during the preparation, release, storage and transportation of the therapeutic radiopharmaceutical).

15   Radical scavengers such as gentisic acid and ascorbic acid have been used to stabilize ^{99m}Tc (DeRosch, et al, WO95/33757) and $^{186/188}$Re (*Anticancer Res.* **1997**, *17*, 1783-1796) radiopharmaceuticals. U.S. Patent 5,393,512 discloses the use of ascorbic acid as a
20   stabilizing agent for ^{186}Re and ^{131}I-labeled antibodies or antibody fragments. Gentisic acid and gentisyl alchohol were also disclosed in U.S. Patent 5,384,113 as stabilizers for radiolabeled peptides. U.S. Patents 5,093,105 and 5,306,482 disclose the use of *p*-
25   aminobenzoic acid, gentisic acid and ascorbic acid as antioxidants for ^{99m}Tc radiopharmaceuticals. U.S. Patent 5,961,955 also discloses a method of ameliorating degradation of radiolabeled peptides, especially radiolabeled proteins such as antibodies, by including
30   PVP (polyvinylpyrrolidinone) as a radioprotectant.

A metalloradiopharmaceutical composition usually includes the BFC-BM conjugate, a buffering agent for pH control, a weak chelating agent to prevent radiometal colloid formation, and a stabilizer to prevent
35   radiolytic degradation of the radiopharmaceutical

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composition during the preparation, release and
transportation of the metalloradiopharmaceutical. The
pH is critical for the success and reproducibility of
the ^{90}Y- or ^{111}In-labeling of biomolecules. Controlling

5   pH (pH 4.0 - 8.0) in the reaction mixture is often
achieved by using 0.1 - 0.5 M ammonium acetate. There
are two purposes in using ammonium acetate for the ^{90}Y-
labeling of biomolecules: (1) pH control during
radiolabeling process and (2) ammonium acetate acts as a

10 transfer ligand for Y^{3+} by forming a weak ^{90}Y-acetate and
preventing the formation of [^{90}Y]colloid. The radiation
stabilizer can be added into the reaction mixture before
(i.e., pre-labeling addition) or after (i.e., post-
labeling addition) the radiolabeling. However, the

15 combination of a buffering agent and a stabilizer often
results in high osmolarity of the radiopharmaceutical
composition.

        Ascorbic acid is known as an antioxidant and has
been used in various pharmaceutical and

20 radiopharmaceutical compositions. Unlike other
buffering agents such as succinic acid and
aminocarboxylates, ascorbic acid contains no amino or
carboxylic groups. One skilled in the art would not
expect to use ascorbic acid as a buffering agent and

25 transfer ligand for the preparation of ^{90}Y or ^{111}In-
labeled biomolecules. Therefore, it is of great
significance, surprising and unexpected that ascorbic
acid and its analogs can serve all three purposes: (1)
as a buffering agent to control the pH of the reaction

30 solution during radiolabeling; (2) as a transfer ligand
to prevent the formation of radiometal colloid; and (3)
as a stabilizer for the radiopharmaceutical composition
during preparation, release, and transportation of the
radiopharmaceutical composition.
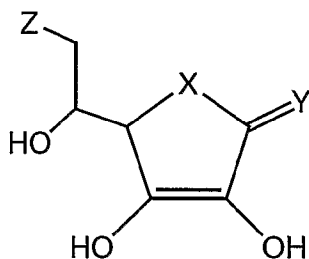
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SUMMARY OF THE INVENTION

There are several advantages using ascorbic acid as a buffering agent. Ascorbic acid has been approved for pharmaceutical and radiopharmaceutical applications.

5   Ascorbic acid has a pKa of 4.2 and has the buffering capacity at pH 3.0 -5.0. At higher concentrations (>50 mg/mL or 0.25 M), it may also have sufficient buffering capacity at the pH range 5.5-6.0. Since ascorbic acid contains two hydroxyl groups, one of which is

10   deprotonable at pH > 4.2, it can also be used as a transfer ligand to prevent the formation of radiometal colloids. Although the use of ascorbic acid as a stabilizer has been disclosed for a variety of diagnostic and therapeutic radiopharmaceutical

15   compositions (see, e.g., Deausch, E. A. et al./U.S. Patent No. 5,384,113/**1995**; Vanderheyden, J.-L., et al./U.S. Patent No. 5,393,512/**1995**; Flanagan, R. J. and Tartaglia, D./U.S. Patent No. 5,093,105/**1992**; Tartaglia, D. and Flanagan, R. J./U.S. Patent No. 5,306,482/**1994**;

20   Shochat, D. et al./U.S. Patent No. 5,961,955/**1999**; and Zamora, P. O. and Merek, M. J./U.S. Patent No. 6,066,309/**2000**), there is no teaching or disclosure on the use of ascorbic acid as a buffering agent and/or as a transfer ligand.

25   If the radiolabeling is performed in the presence of ascorbic acid at pH 4 - 6, there is no need for a buffering agent such as ammonium acetate in the reaction solution because ascorbic acid has sufficient buffering capacity at this pH range. In doing so, it will

30   eliminate possible side effect from ammonium cation, a well-known vasodilator, particularly at high concentrations, and will result in dramatic reduction of the osmolarity of the radiopharmaceutical composition.

DETAILED DESCRIPTION OF THE INVENTION

[1] One embodiment of the present invention provides a
radiopharmaceutical composition comprising a
5   radiolabeled chelator-biomolecule conjugate of the
formula $M-Ch-Ln-(BM)_m$; and an amount of a compound of
formula (I):

10   (I)

or a pharmaceutically acceptable salt thereof,

wherein

          X is O, NR^1, or CHR^1;

15   Y is O or S;

          Z is hydroxyl or halogen;

          R^1 is selected from: (C_1-C_{10})alkyl substituted with
0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted
20   with 0-5 R^2; and

          R^2 is independently selected at each occurrence
from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and
SO_3H;

wherein the amount of the compound of formula (I)
is effective to: (1) stabilize the radiolabeled
chelator-biomolecule conjugate of the formula M-Ch-Ln-
$(BM)_m$ against radiation induced degradation and to at
5   least one of (2) control the pH of the
radiopharmaceutical composition and (3) prevent
radiometal colloid formation.

[2]   Another embodiment of the present invention
10   provides a radiopharmaceutical composition of embodiment
1 wherein the amount is effective to: (1) stabilize the
radiopharmaceutical against radiation induced
degradation and (2) control the pH of the
radiopharmaceutical.

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[3]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the amount is effective to: (1) stabilize
the radiopharmaceutical against radiation induced
20   degradation and (2) prevent radiometal colloid
formation.

[4]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
25   [1] wherein the amount is effective to: (1) stabilize
the radiopharmaceutical against radiation induced
degradation, (2) control the pH of the
radiopharmaceutical and (3) prevent radiometal colloid
formation.

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[5]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein X is O.

5   [6]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein Y is O.

[7]   Another embodiment of the present invention
10   provides a radiopharmaceutical composition of embodiment
[1] wherein Z is hydroxyl.

[8]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
15   [1] wherein m is 1 to about 5.

[9]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein m is 1 or 2.

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[10]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein m is 1.

25   [11]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein m is 1 to about 5; X is O; and Y is O.

[12] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein m is 1 or 2; X is O; Y is O; and Z is
hydroxyl.

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[13] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein m is 1; X is O; Y is O; and Z is hydroxyl.

10   [14] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the concentration of the compound of formula
(I) is about 2 mg/mL to about 200 mg/mL.

15   [15] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the metallic radioisotope is present at a
level of about 10 mCi to about 2000 mCi.

20   [16] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the metallic radioisotope is present at a
concentration of greater than about 5 mCi/mL.

25   [17] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the radiolabeled chelator-biomolecule
conjugate of the formula M-Ch-Ln-(BM)$_m$ is a diagnostic
radiopharmaceutical.

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[18]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the radiolabeled chelator-biomolecule
5   conjugate of the formula M-Ch-Ln-(BM)$_m$ is a therapeutic
radiopharmaceutical.

[19]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
10   [1] wherein the biomolecule is an antibody.

[20]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the biomolecule is an antibody fragment.

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[21]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the biomolecule is a peptide.

20   [22]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the biomolecule is a peptidomimetic.

[23]   Another embodiment of the present invention
25   provides a radiopharmaceutical composition of embodiment
[1] wherein the biomolecule is a non-peptide.

[24]   Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment

[1] wherein the biomolecule is a cyclic IIb/IIIa
receptor antagonist; an RGD containing peptide; a
fibrinogen receptor antagonist; a IIb/IIIa receptor
ligand; a ligand for the polymerization site of fibrin;
5   a laminin derivative; a ligand for fibrinogen; a
thrombin ligand; an oligopeptide that corresponds to the
IIIa protein; a hirudin-based peptide; a IIb/IIIa
receptor ligand; a thrombus, platelet binding, or
atherosclerotic plaque binding peptide; a fibrin binding
10   peptide; a hirudin-based peptide; a fibrin binding
protein; a guanine derivative that binds to the IIb/IIIa
receptor; a tyrosine derivative; a leukocyte binding
peptide; a chemotactic peptide; a leukostimulatory
agent; an LTB4 antagonist; a somatostatin analog; a
15   selectin binding peptide; a biological-function domain;
a platelet factor 4 or growth factor; a compound that
binds to a receptor that is expressed or upregulated in
angiogenic tumor vasculature; a peptide, polypeptide or
peptidomimetic that binds with high affinity to the
20   receptors VEGF receptors Flk-1/KDR, Flt-1, or
neuropilin-1; a peptide, polypeptide or peptidomimetic
that binds to $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha 5\beta 1$, $\alpha 4\beta 1$, $\alpha 1\beta 1$, or $\alpha 2\beta 2$; a
compound that interacts with receptor tyrosine kinases;
a protein, antibody, antibody fragment, peptide,
25   polypeptide, or peptidomimetic that binds to receptors
or binding sites on a tissue, organ, enzyme or fluid; a
ß-amyloid protein that has been demonstrated to
accumulate in patients with Alzheimer's disease; an
atrial naturetic factor derived peptide that binds to
30   myocardial or renal receptor; an antimyosin antibody
that binds to areas of infarcted tissue; or a
nitroimidazole derivative that localizes in hypoxic
areas in vivo.

[25] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the chelator is a cyclic or acyclic

5   polyaminocarboxylate, a diaminedithiol, a
triamidemonothiol, a monoaminemonoamidedithiol, a
monoaminediamidemonothiol, a diaminedioxime, or a
hydrazine.

10 [26] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the chelator is tetradentate, with donor
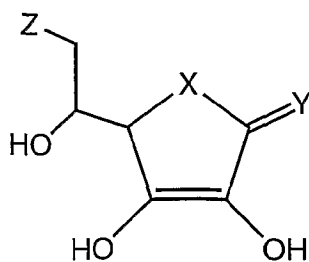atoms selected from nitrogen, oxygen and sulfur.

15 [27] Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the chelator is
diethylenetriaminepentaacetic acid (DTPA); 1,4,7,10-
tetraazazcyclododecane-1,4,7,10-tetraacetic acid (DOTA);

20 1,4,8,11-tetraazazcyclotetradecane-1,4,8,11-tetraacetic
acid (TETA); 1,4,7,10-tetraazazcyclododecane-1,4,7-
triacetic acid (DO3A); 2-Benzyl-1,4,7,10-
tetraazazcyclododecane-1,4,7,10-tetraacetic acid (2-Bz-
DOTA); alpha-(2-phenethyl)-1,4,7,10-

25 tetraazazcyclododecane-1-acetic-4,7,10-
tris(methylacetic) acid; 2-benzyl-
cyclohexyldiethylenetriaminepentaacetic acid; 2-benzyl-
6-methyl-diethylenetriaminepentaacetic acid; or 6,6"-
bis[N,N,N",N"-tetra(carboxymethyl)aminomethyl]-4'-(3-

30 amino-4-methoxyphenyl)-2,2':6',2"-terpyridine.

[28]　Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the metallic radioisotope is ^{177}Lu, ^{149}Pm,

5　^{153}Sm, ^{166}Ho, ^{90}Y, ^{111}In, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{99m}Tc, ^{117m}Sn, ^{203}Pb,
^{177}Lu, ^{47}Sc, ^{109}Pd, ^{105}Rh, ^{186}Re, ^{188}Re, ^{60}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu,
^{97}Ru, or ^{212}Bi.

[29]　Another embodiment of the present invention
10　provides a radiopharmaceutical composition of embodiment
[1] wherein the metallic radioisotope is ^{99m}Tc, ^{117m}Sn,
^{111}In, ^{203}Pb, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{177}Lu, ^{149}Pm, ^{153}Sm, ^{166}Ho,
^{47}Sc, ^{109}Pd, ^{105}Rh, ^{186}Re, ^{188}Re, ^{60}Cu, ^{62}Cu, ^{64}Cu or ^{67}Cu.

15　[30]　Another embodiment of the present invention
provides a radiopharmaceutical composition of embodiment
[1] wherein the metallic radioisotope is ^{111}In, ^{90}Y, or
^{177}Lu.

20　[31]　Another embodiment of the present invention
provides a radiopharmaceutical composition comprising a
radiolabeled chelator-biomolecule conjugate of the
formula M-Ch-Ln-(BM)$_m$; and a compound of formula (I):

(I)

or a pharmaceutically acceptable salt thereof;

wherein

M is a metallic radioisotope;

Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule;

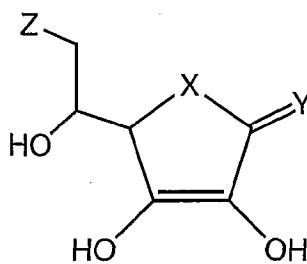m is 1 to about 10;

X is O, NR^1, or CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with 0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2;

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and SO_3H;

provided the radiopharmaceutical composition does not comprise an additional buffering agent or an additional chelating agent.

[32] Another embodiment of the present invention provides a method for buffering a radiopharmaceutical comprising contacting the radiopharmaceutical with an amount of a compound of formula (I):

22

(I)

5   or a pharmaceutically acceptable salt thereof,

wherein

       X is O, NR^1, or CHR^1;

       Y is O or S;

       Z is hydroxyl or halogen;

10   R^1 is selected from: (C_1-C_{10})alkyl substituted with
0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted
with 0-5 R^2;

       R^2 is independently selected at each occurrence
15   from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and
SO_3H;

       wherein the amount is effective to control the pH
of the radiopharmaceutical.

20   [33] Another embodiment of the present invention
provides a method of embodiment [32] wherein the
radiopharmaceutical is a radiolabeled chelator-
biomolecule conjugate of the formula $M-Ch-Ln-(BM)_m$;

wherein

    M is a metallic radioisotope;

   Ch is a metal chelator;

    Ln is an optional linking group;

5   BM is a biomolecule; and

    m is 1 to about 10.

[34] Another embodiment of the present invention
provides a method of embodiment [32] wherein the
10 buffering agent controls the pH of the
radiopharmaceutical during at least one of the
preparation, release, storage, and transportation of the
radiopharmaceutical.

15 [35] Another embodiment of the present invention
provides a method for chelating a radiopharmaceutical
comprising contacting the radiopharmaceutical with an
amount of a compound of formula (I):

(I)

20
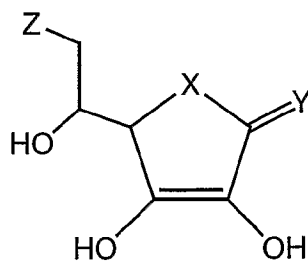
or a pharmaceutically acceptable salt thereof,

wherein

    X is O, NR^1, or CHR^1;

    Y is O or S;

    Z is hydroxyl or halogen;

5   R^1 is selected from: (C_1-C_{10})alkyl substituted with 0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2;

R^2 is independently selected at each occurrence from: 10 NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and SO_3H;

    wherein the amount is effective to prevent radiometal colloid formation.

[36]   Another embodiment of the present invention 15 provides a method of embodiment [35] wherein the radiopharmaceutical is a radiolabeled chelator-biomolecule conjugate of the formula M-Ch-Ln-$(BM)_m$; wherein

    M is a metallic radioisotope;

20   Ch is a metal chelator;

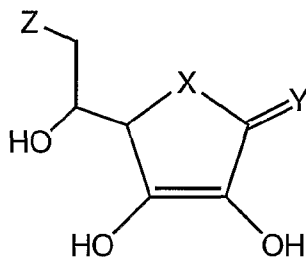   Ln is an optional linking group;

    BM is a biomolecule; and

    m is 1 to about 10.

25 [37]   Another embodiment of the present invention provides a method of embodiment [35] wherein the chelating agent prevents radiometal colloid formation

during at least one of the preparation, release, storage, and transportation of the radiopharmaceutical.

[38]   Another embodiment of the present invention provides a method for stabilizing a radiopharmaceutical against radiation induced degradation and at least one of (1) controlling the pH of the radiopharmaceutical and (2) preventing radiometal colloid formation; comprising contacting the radiopharmaceutical with an amount of a compound of formula (I):

(I)

or a pharmaceutically acceptable salt thereof, wherein

X is O, NR^1, or CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with 0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2;

R^2 is independently selected at each occurrence from: NH$_2$, OH, CO$_2$H, C(=O)NH$_2$, NHC(=NH)NH$_2$, PO$_3$H$_2$, and SO$_3$H;

wherein the amount is effective to: (1) stabilize
5   the radiopharmaceutical against radiation induced degradation and to at least one of (2) control the pH of the radiopharmaceutical and (3) prevent radiometal colloid formation.

10   [39]   Another embodiment of the present invention provides a method of embodiment [38] wherein the amount is effective to: (1) stabilize the radiopharmaceutical against radiation induced degradation and (2) control the pH of the radiopharmaceutical.

15

[40]   Another embodiment of the present invention provides a method of embodiment [38] wherein the amount is effective to: (1) stabilize the radiopharmaceutical against radiation induced degradation and (2) prevent
20   radiometal colloid formation.

[41]   Another embodiment of the present invention provides a method of embodiment [38] wherein the amount is effective to: (1) stabilize the radiopharmaceutical
25   against radiation induced degradation, (2) control the pH of the radiopharmaceutical and (3) prevent radiometal colloid formation.

[42]   Another embodiment of the present invention provides a method of embodiment [38] wherein the radiopharmaceutical is a radiolabeled chelator-biomolecule conjugate of the formula M-Ch-Ln-(BM)$_m$

5   wherein

M is a metallic radioisotope;

Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule; and

10   m is 1 to about 10.

[43]   Another embodiment of the present invention provides a method of embodiment [38] wherein the amount is effective to: stabilize the radiopharmaceutical

15   against radiation induced degradation and to at least one of (2) control the pH of the radiopharmaceutical and (3) prevent radiometal colloid formation; during at least one of the preparation, release, storage, and transportation of the radiopharmaceutical.

20

[44]   Another embodiment of the present invention provides a method for preparing a stable radiopharmaceutical composition comprising contacting a radiolabeled chelator-biomolecule conjugate of the

25   formula M-Ch-Ln-(BM)$_m$; and an amount of a compound of formula (I):

(I)

or a pharmaceutically acceptable salt thereof,

wherein

X is O, NR^1, or CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with 0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2; and

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and SO_3H;

wherein the amount of the compound of formula (I) is effective to: (1) stabilize the radiolabeled chelator-biomolecule conjugate of the formula M-Ch-Ln-$(BM)_m$ against radiation induced degradation and to at least one of (2) control the pH of the radiopharmaceutical composition and (3) prevent radiometal colloid formation.

[45]   Another embodiment of the present invention
provides a kit comprising a sealed vial comprising a
predetermined quantity of a radiolabeled chelator-
biomolecule conjugate of the formula $M\text{-}Ch\text{-}Ln\text{-}(BM)_m$, and
5   an amount of a compound of formula (I):

(I)

10   or a pharmaceutically acceptable salt thereof,
wherein

   M is a metallic radioisotope;

   Ch is a metal chelator;

   Ln is an optional linking group;

15   BM is a biomolecule;

   m is 1 to about 10;

   X is selected from O, NR^1, and CHR^1;

   Y is O or S;
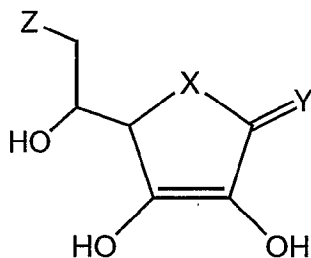
   Z is hydroxyl or halogen;

20   R^1 is selected from: $(C_1\text{-}C_{10})$alkyl substituted with
0-5 R^2, $(C_3\text{-}C_{10})$cycloalkyl substituted with 0-5 R^2, $(C_2\text{-}$

C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2; and

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and

5   SO_3H;

wherein the amount is effective to: (1) stabilize the radiolabeled chelator-biomolecule conjugate of the formula $M-Ch-Ln-(BM)_m$ against radiation induced degradation and to at least one of (2) control the pH of

10   the radiopharmaceutical and (3) prevent radiometal colloid formation.

[46]   Another embodiment of the present invention provides a kit comprising (a) a first vial comprising a

15   predetermined quantity of a radiolabeled chelator-biomolecule conjugate of the formula $M-Ch-Ln-(BM)_m$; and an amount of a compound of formula (I):

20   (I)

or a pharmaceutically acceptable salt thereof, wherein

M is a metallic radioisotope;

Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule;

5   m is 1 to about 10;

X is selected from O, NR^1, and CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

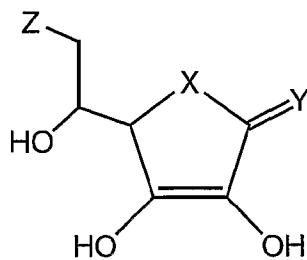R^1 is selected from: (C_1-C_{10})alkyl substituted with

10   0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2; and

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and

15   SO_3H;

wherein the amount is effective to: (1) stabilize the radiopharmaceutical against radiation induced degradation and to at least one of (2) control the pH of the radiopharmaceutical and (3) prevent radiometal

20   colloid formation; and

       (a)   a second vial comprising a pharmaceutically
                acceptable carrier or diluent.

[47]   Another embodiment of the present invention

25   provides a novel compound of formula (I):

(I)

or a pharmaceutically acceptable salt thereof,

5   wherein

X is O, NR^1, or CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with

10   0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2; and

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and

15   SO_3H.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various

20   features of the invention which are for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

DEFINITIONS

The radiopharmaceutical compositions of the present
invention are comprised of a radioisotope-chelator-
biomolecule conjugate, ascorbic acid or an analog
5   thereof, and other optional pharmaceutical excipients.
The target-specific radiopharmaceuticals comprised of a
gamma ray-emitting isotope or positron-emitting isotope
are useful as imaging agents. The radiopharmaceuticals
comprised of a beta particle, alpha particle or Auger
10   electron-emitting isotope are useful as therapeutic
radiopharmaceuticals. The metallic radioisotope is
chelated by the BFC attached directly or optionally via
a linker to one or more biomolecules. Biomolecules are
proteins, antibodies, antibody fragments, single-chain
15   antibodies, polypeptides, oligonucleotides, peptides,
peptidomimetics or non-peptides. Preferably, the
biomolecules are peptides, peptidomimetics, and non-
peptides of less than 10,000 g/mol molecular weight.
The ascorbic acid or analog thereof serves three
20   purposes: it acts as a buffering agent for pH control
during radiolabeling, it acts as a chelating agent to
prevent radiometal colloid formation, and it acts as a
stabilizer to provide protection against radiation
induced degradation of the radiolabeled compound.
25   Metallic radioisotopes that emit alpha particles, beta
particles, gamma rays, positrons, or Auger electrons
useful for imaging or therapy include ^{99m}Tc, ^{117m}Sn, ^{111}In,
^{97}Ru, ^{203}Pb, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{177}Lu, ^{149}Pm, ^{153}Sm, ^{166}Ho,
^{212}Bi, ^{47}Sc, ^{109}Pd, ^{105}Rh, ^{186}Re, ^{188}Re, ^{60}Cu, ^{62}Cu, ^{64}Cu and
30   ^{67}Cu.

Examples of preferred biomolecules that may be part
of the metallic radioisotope-chelator-biomolecule (M-
BFC-BM) conjugate include the following.

For the diagnosis of thromboembolic disorders or
35   atherosclerosis, BM is selected from the group including

34

the cyclic IIb/IIIa receptor antagonist compounds
described in U.S. Patent 5,879,657; the RGD containing
peptides described in U.S. Patents 4,578,079, 4,792,525,
the applications PCT US88/04403, PCT US89/01742, PCT
5   US90/03788, PCT US91/02356 and by Ojima et. al. 204th
Meeting of the Amer. Chem. Soc. **1992**, Abstract 44; the
peptides that are fibrinogen receptor antagonists
described in European Patent Applications 90202015.5,
90202030.4, 90202032.2, 90202032.0, 90311148.2,
10   90311151.6, 90311537.6, the specific binding peptides
and polypeptides described as IIb/IIIa receptor ligands,
ligands for the polymerization site of fibrin, laminin
derivatives, ligands for fibrinogen, or thrombin ligands
in PCT WO 93/23085 (excluding the technetium binding
15   groups); the oligopeptides that correspond to the IIIa
protein described in PCT WO90/00178; the hirudin-based
peptides described in PCT WO90/03391; the IIb/IIIa
receptor ligands described in PCT WO90/15818; the
thrombus, platelet binding or atherosclerotic plaque
20   binding peptides described in PCT WO92/13572 (excluding
the technetium binding group) or GB 9313965.7; the
fibrin binding peptides described in U.S. Patents
4,427,646 and 5,270,030; the hirudin-based peptides
described in U.S. Patent 5,279,812; or the fibrin
25   binding proteins described in U.S. Patent 5,217,705; the
guanine derivatives that bind to the IIb/IIIa receptor
described in U.S. Patent 5,086,069; or the tyrosine
derivatives described in European Patent Application
0478328A1, and by Hartman et. al., *J. Med. Chem.* **1992**,
30   *35*, 4640; or oxidized low density lipoprotein (LDL).

      For the diagnosis of infection, inflammation or
transplant rejection, BM is selected from the group
including the leukocyte binding peptides described in
PCT WO93/17719 (excluding the technetium binding group),
35   PCT WO92/13572 (excluding the technetium binding group)

or U.S. Ser. No. 08-140000; the chemotactic peptides described in Eur. Pat. Appl. 90108734.6 or A. Fischman et. al., Semin. Nuc. Med., 1994, 24, 154; the leukostimulatory agents described in U.S. Patent

5   5,277,892; or the LTB4 antagonists described in co-pending U.S.S.N. 08/943,659.

For the diagnosis of cancer, BM is selected from the group of somatostatin analogs described in UK Application 8927255.3 or PCT WO94/00489, the selectin

10   binding peptides described in PCT WO94/05269, the biological-function domains described in PCT WO93/12819, Platelet Factor 4 or the growth factors (PDGF, VEGF, EGF, FGF, TNF MCSF or the interleukins Il1-8).

BM may also be a compound that binds a receptor

15   that is expressed or upregulated in angiogenic tumor vasculature. For targeting the VEGF receptors, Flk-1/KDR, Flt-1, and neuropilin-1, the targeting moieties are comprised of peptides, polypeptides or peptidomimetics that bind with high affinity to the

20   receptors. For example, peptides comprised of a 23 amino acid portion of the C-terminal domain of VEGF have been synthesized which competitively inhibit binding of VEGF to VEGFR (Soker, et. al., J. Biol. Chem., 1997, 272, 31582-8). Linear peptides of 11 to 23 amino acid

25   residues that bind to the basic FGF receptor (bFGFR) are described by Cosic et. al., Mol. and Cell. Biochem., 1994, 130, 1-9. A preferred linear peptide antagonist of the bFGFR is the 16 amino acid peptide, Met-Trp-Tyr-Arg-Pro-Asp-Leu-Asp-Glu-Arg-Lys-Gln-Gln-Lys-Arg-Glu.

30   Gho et. al. (Cancer Research, 1997, 57, 3733-40) describe the identification of small peptides that bind with high affinity to the angiogenin receptor on the surface of endothelial cells. A preferred peptide is Ala-Gln-Leu-Ala-Gly-Glu-Cys-Arg-Glu-Asn-Val-Cys-Met-Gly-

35   Ile-Glu-Gly-Arg, in which the two Cys residues form an

intramolecular disulfide bond. Yayon et. al. (Proc. Natl. Acad. Sci, USA, 1993, 90, 10643-7) describe other linear peptide antagonists of FGFR, identified from a random phage-displayed peptide library. Two linear

5   octapeptides, Ala-Pro-Ser-Gly-His-Tyr-Lys-Gly and Lys-Arg-Thr-Gly-Gln-Tyr-Lys- Leu are preferred for inhibiting binding of bFGF to it receptor.

Targeting moieties for integrins expressed in tumor vasculature include peptides, polypeptides and

10   peptidomimetics that bind to $\alpha v \beta 3$, $\alpha v \beta 5$, $\alpha 5 \beta 1$, $\alpha 4 \beta 1$, $\alpha 1 \beta 1$, and $\alpha 2 \beta 2$. Pierschbacher and Rouslahti (*J. Biol. Chem.* **1987**, *262*, 17294-17298) describe peptides that bind selectively to $\alpha 5 \beta 1$ and $\alpha v \beta 3$. U.S. 5,536,814 describe peptides that bind with high affinity to the

15   integrin $\alpha 5 \beta 1$. Burgess and Lim (*J. Med. Chem.* **1996**, *39*, 4520-4526) disclose the synthesis three peptides that bind with high affinity to $\alpha v \beta 3$: cyclo[Arg-Gly-Asp-Arg-Gly-Asp], cyclo[Arg-Gly-Asp-Arg-Gly-D-Asp] and the linear peptide Arg-Gly-Asp-Arg-Gly-Asp. U.S. 5,770,565

20   and U.S. 5,766,591 disclose peptides that bind with high affinity to $\alpha v \beta 3$. U.S. 5,767,071 and U.S. 5,780,426, disclose cyclic peptides that have an exocyclic Arg amino acid that have high affinity for $\alpha v \beta 3$. Srivatsa et. al., (*Cardiovascular Res.* **1997**, *36*, 408-428)

25   describe the cyclic peptide antagonist for $\alpha v \beta 3$, cyclo[Ala-Arg-Gly-Asp-Mamb]. Tran et. al., (*Bioorg. Med. Chem. Lett.* **1997**, *7*, 997-1002) disclose the cyclic peptide cyclo[Arg-Gly-Asp-Val-Gly-Ser-BTD-Ser-Gly-Val-Ala] that binds with high affinity to avB3. Arap et.

30   al. (*Science* **1998**, *279*, 377-380) describe cyclic peptides that bind to $\alpha v \beta 3$ and $\alpha v \beta 5$, Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys, and cyclo[Cys-Asn-Gly-Asp-Cys]. Corbett et. al. (*Biorg. Med. Chem. Lett.* **1997**, *7*, 1371-1376) describe a series of $\alpha v \beta 3$ selective

35   peptidomimetics. And Haubner et. al., (*Angew. Chem. Int.*

Ed. Engl. **1997**, *36*, 1374-1389) disclose peptides and
peptidomimetic αvβ3 antagonists obtained from peptide
libraries.

Alternative targeting moieties for tumor
5   vasculature include compounds that interact with
receptor tyrosine kinases. Receptor tyrosine kinases
(TKs) are membrane proteins, which play a key role in
the transduction of mitogenic signals across the cell to
the nucleus (Rewcastle, G. W. et al *J. Med. Chem.* **1995**,
10 *38*, 3482-3487; Thompson, A. M. et al *J. Med. Chem.*
1997, *40*, 3915-3925). Of the many TKs that have been
identified and characterized, those of the epidermal
growth factor receptor (EGFR) family are particularly
important, and have been implicated in a variety of
15 ectopic cell proliferative processes. The over-
expression of human EGF receptor is greatly amplified in
several human tumors (Fry, D. W. *Exp. Opin. Invest.*
Drugs **1994**, *3*, 577-595; Jardines, L. et al *Pathobiology*
1993, *61*, 268-282), accompanied by an
20 overphosphorylation of their protein targets. This
increased phosphorylation of substrate tyrosine residues
by oncogenic TK proteins is an essential step in the
neoplastic transformation. Consequently, there has been
great interest in developing inhibitors of TKs (TKIs) as
25 anticancer drugs (Burke, T. R. Jr. *Drugs Future* **1992**
17, 119-131; Chang, C. J. and Geahlen, R. *J. Nat. Prod.*
1992, *55*, 1529-1560). The over-expression of EGF
receptors in tumor cells also provides the foundation
for the development of diagnostic and therapeutic
30 radiopharmaceuticals by attaching a chelator and a
radionuclide onto the TK receptor ligand (tyrosine
kinase inhibitor).

BM may also represent proteins, antibodies,
antibody fragments, peptides, polypeptides, or
35 peptidomimetics that bind to receptors or binding sites

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on other tissues, organs, enzymes or fluids. Examples
include the ß-amyloid proteins that have been
demonstrated to accumulate in patients with Alzheimer's
disease, atrial naturetic factor derived peptides that
5   bind to myocardial and renal receptors, antimyosin
antibodies that bind to areas of infarcted tissues, or
nitroimidazole derivatives that localize in hypoxic
areas in vivo.

       The linking group L_n can serve several roles.
10   First it provides a spacing group between the metal
chelator, C_h, and the one or more of the biomolecules,
BM, so as to minimize the possibility that the metal
chelate $M-C_h$ will interfere with the interaction of the
biomolecule with its biological target. The necessity
15   of incorporating a linking group in a reagent is
dependent on the identity of BM and $M-C_h$. If metal
chelate $M-C_h$ cannot be attached to BM without
substantially diminishing its affinity for its
biological target, then a linking group is used. A
20   linking group also provides a means of independently
attaching multiple biomolecules to one group that is
attached to $M-C_h$.

       The linking group also provides a way of
incorporating a pharmacokinetic modifier into the
25   pharmaceuticals of the present invention. The
pharmacokinetic modifier serves to direct the
biodistibution of the injected pharmaceutical other than
by the interaction of the biomolecules, BM, with the
biological target. A wide variety of functional groups
30   can serve as pharmacokinetic modifiers, including, but
not limited to, carbohydrates, polyalkylene glycols,
peptides or other polyamino acids, and cyclodextrins.
The modifiers can be used to enhance or decrease
hydrophilicity and to enhance or decrease the rate of

blood clearance. The modifiers can also be used to direct the route of elimination of the pharmaceuticals.

The metal chelator or bonding moiety, C_h, is selected to form stable complexes with the metal ion
5   chosen for the particular application. Chelators or bonding moieties for diagnostic radiopharmaceuticals are selected to form stable chelates with the radioisotopes that have imageable gamma ray or positron emissions.

Chelators for technetium and rhenium isotopes are
10   selected from diaminedithiols, triamidemonothiols, monoaminemonoamidedithiols, monoaminediamidemonothiols, diaminedioximes, and hydrazines. The chelators are generally tetradentate with donor atoms selected from nitrogen, oxygen and sulfur. Preferred reagents are
15   comprised of chelators having amine nitrogen and thiol sulfur donor atoms and hydrazine bonding units. The thiol sulfur atoms and the hydrazines may bear a protecting group which can be displaced either prior to using the reagent to synthesize a radiopharmaceutical or
20   preferably in situ during the synthesis of the radiopharmaceutical.

Exemplary thiol protecting groups include those listed in Greene and Wuts, "Protective Groups in Organic Synthesis" John Wiley & Sons, New York (1991), the
25   disclosure of which is hereby incorporated by reference. Any thiol protecting group known in the art can be used. Examples of thiol protecting groups include, but are not limited to, the following: acetamidomethyl, benzamidomethyl, 1-ethoxyethyl, benzoyl, and
30   triphenylmethyl.

Exemplary protecting groups for hydrazine bonding units are hydrazones which can be aldehyde or ketone hydrazones having substituents selected from hydrogen, alkyl, aryl and heterocycle. Particularly preferred

hydrazones are described in co-pending U.S.S.N. 08/476,296 the disclosure of which is herein incorporated by reference in its entirety.

5   The hydrazine-bonding unit when bound to a metal radionuclide is termed a hydrazido, or diazenido group and serves as the point of attachment of the radionuclide to the remainder of the radiopharmaceutical. A diazenido group can be either terminal (only one atom of the group is bound to the
10   radionuclide) or chelating. In order to have a chelating diazenido group at least one other atom of the group must also be bound to the radionuclide. The atoms bound to the metal are termed donor atoms.

Chelators for chelation of radioniclides, including
15   ^{111}In, ^{86}Y, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{62}Cu, ^{64}Cu and ^{67}Cu, are selected from polyaminocarboxylates, such as diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-tetraazazcyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,8,11-tetraazazcyclotetradecane-1,4,8,11-tetraacetic
20   acid (TETA),1,4,7,10-tetraazazcyclododecane-1,4,7-triacetic acid (DO3A), 2-Benzyl-1,4,7,10-tetraazazcyclododecane-1,4,7,10-tetraacetic acid (2-Bz-DOTA), alpha-(2-phenethyl)-1,4,7,10-tetraazazcyclododecane-1-acetic-4,7,10-
25   tris(methylacetic) acid, 2-benzyl-cyclohexyldiethylenetriaminepentaacetic acid, 2-benzyl-6-methyl-diethylenetriaminepentaacetic acid, and 6,6"-bis[N,N,N",N"-tetra(carboxymethyl)aminomethyl]-4'-(3-amino-4-methoxyphenyl)-2,2':6',2"-terpyridine.
30   Procedures for synthesizing these chelators that are not commercially available can be found in Brechbiel, M. and Gansow, O., *J. Chem. Soc. Perkin Trans.* **1992**, *1*, 1175; Brechbiel, M. and Gansow, O., *Bioconjugate Chem.* **1991**, *2*, 187; Deshpande, S., et. al., *J. Nucl. Med.* **1990**, *31*,
35   473; Kruper, J., U.S. Patent 5,064,956, and Toner, J.,

U.S. Patent 4,859,777, the disclosures of which are
hereby incorporated by reference in their entirety.

Chelators or bonding moieties for therapeutic
radiopharmaceuticals are selected to form stable
5 complexes with the radioisotopes that have alpha
particle, beta particle, Auger or Coster-Kronig electron
emissions. Chelators for rhenium, copper, palladium,
platinum, iridium, rhodium, silver and gold isotopes are
selected from diaminedithiols,
10 monoaminemonoamidedithiols, triamidemonothiols,
monoaminediamidemonothiols, diaminedioximes, and
hydrazines. Chelators for yttrium, bismuth, and the
lanthanide isotopes are selected from cyclic and
acyclic polyaminocarboxylates, including
15 diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-
tetraazazcyclododecane-1,4,7,10-tetraacetic acid (DOTA),
1,4,8,11-tetraazazcyclotetradecane-1,4,8,11-tetraacetic
acid (TETA),1,4,7,10-tetraazazcyclododecane-1,4,7-
triacetic acid (DO3A), 2-Benzyl-1,4,7,10-
20 tetraazazcyclododecane-1,4,7,10-tetraacetic acid (2-Bz-
DOTA), alpha-(2-phenethyl)-1,4,7,10-
tetraazazcyclododecane-1-acetic-4,7,10-
tris(methylacetic) acid, 2-benzyl-
cyclohexyldiethylenetriaminepentaacetic acid, 2-benzyl-
25 6-methyl-diethylenetriaminepentaacetic acid, and 6,6"-
bis[N,N,N",N"-tetra(carboxymethyl)aminomethyl)-4'-(3-
amino-4-methoxyphenyl)-2,2':6',2"-terpyridine.

The integrity of a radiopharmaceutical is measured
by the radiochemical purity (RCP) of the radiolabeled
30 compound using ITLC or more preferably HPLC. The
advantage of using HPLC is that radio-impurities caused
by radiolytic degradation can be separated from the
radiopharmaceutical under optimized chromatographic
conditions. Improved stability over time for
35 radiopharmaceutical compositions of this invention can

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be demonstrated by determining the change in RCP of the
radiolabeled compound in samples taken at representative
time points. The radiopharmaceutical compositions of
this invention are effective in maintaining the long-
5   term stability of samples that have been frozen, thawed,
and re-tested up to 5 days post-labeling.

The initial RCP of a radiopharmaceutical is largely
dependent on radiolabeling conditions such as pH,
heating temperature and time. Once a
10   radiopharmaceutical is prepared in high yield, the
ability of an antioxidant to stabilize a
radiopharmaceutical composition is measured by the RCP
change over a certain period of time.

Therapeutic radiopharmaceutical compositions are
15   preferably stored at low temperature to avoid extensive
radiolysis during release and transportation. The
amount of the stabilizer used in the therapeutic
radiopharmaceutical composition and storage temperature
during release and transportation may be adjusted
20   according to the sensitivity of a specific radiolabeled
compound towards radiolytic decomposition.

Ascorbic acid is known as vitamin C, and is a
commonly used antioxidant to prevent radiolytic
decomposition of ^{99m}Tc and $^{186/188}Re$ radiopharmaceuticals
25   (WO95/33757; *Anticancer Res.* **1997**, *17*, 1783-1796; US
patent 5,093,105, and US patent 5,306,482) or
radiolabeled peptides (US patent 5,393,512; US patent
5,384,113 and US patent 5,961,955). Ascorbic acid is
readily available GRAS (generally recognized as safe)
30   substance often used in pharmaceutical compositions and
other formulations used for biological purpose and may
be used at levels as high as 200 mg/mL of the final
formulation. The major advantages of using ascorbic
acid or its analogs in a radiopharmaceutical composition
35   disclosed in this invention include: (1) the

radiopharmaceutical can be prepared in high yield
(>90%); (2) the radiometal colloid formation is minimal
(<1%); and (3) the radiopharmaceutical composition can
be stored for several days, while maintaining the RCP
5   (>90%) of the radiopharmaceutical.

The compounds herein described may have asymmetric
centers. Compounds of the present invention containing
an asymmetrically substituted atom may be isolated in
optically active or racemic forms. It is well known in
10 the art how to prepare optically active forms, such as
by resolution of racemic forms or by synthesis from
optically active starting materials. Many geometric
isomers of olefins, C=N double bonds, and the like can
also be present in the compounds described herein, and
15 all such stable isomers are contemplated in the present
invention. Cis and trans geometric isomers of the
compounds of the present invention are described and may
be isolated as a mixture of isomers or as separated
isomeric forms. All chiral, diastereomeric, racemic
20 forms and all geometric isomeric forms of a structure
are intended, unless the specific stereochemistry or
isomeric form is specifically indicated. All processes
used to prepare compounds of the present invention and
intermediates made therein are considered to be part of
25 the present invention.

The term "substituted," as used herein, means that
any one or more hydrogens on the designated atom is
replaced with a selection from the indicated group,
provided that the designated atom's normal valency is
30 not exceeded, and that the substitution results in a
stable compound. When a substitent is keto (i.e., =O),
then 2 hydrogens on the atom are replaced. Keto
substituents are not present on aromatic moieties. When
a ring system (e.g., carbocyclic or heterocyclic) is
35 said to be substituted with a carbonyl group or a double

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bond, it is intended that the carbonyl group or double
bond be part (i.e., within) of the ring.

The present invention is intended to include all
isotopes of atoms occurring in the present compounds.
5   Isotopes include those atoms having the same atomic
number but different mass numbers. By way of general
example and without limitation, isotopes of hydrogen
include tritium and deuterium. Isotopes of carbon
include C-13 and C-14.

10   When any variable (e.g., R^5) occurs more than one
time in any constituent or formula for a compound, its
definition at each occurrence is independent of its
definition at every other occurrence. Thus, for
example, if a group is shown to be substituted with 0-2
15   R^5, then said group may optionally be substituted with
up to two R^5 groups and R^5 at each occurrence is
selected independently from the definition of R^9. Also,
combinations of substituents and/or variables are
permissible only if such combinations result in stable
20   compounds.

When a bond to a substituent is shown to cross a
bond connecting two atoms in a ring, then such
substituent may be bonded to any atom on the ring. When
a substituent is listed without indicating the atom via
25   which such substituent is bonded to the rest of the
compound of a given formula, then such substituent may
be bonded via any atom in such substituent.
Combinations of substituents and/or variables are
permissible only if such combinations result in stable
30   compounds.

As used herein, "alkyl" is intended to include both
branched and straight-chain saturated aliphatic
hydrocarbon groups having the specified number of carbon
atoms. Examples of alkyl include, but are not limited

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to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, and s-pentyl. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, or cyclopentyl. "Alkenyl" is
5   intended to include hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl and propenyl.

10   "Halo" or "halogen" as used herein refers to fluoro, chloro, bromo, and iodo; and "counterion" is used to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, and sulfate.

15   As used herein, "carbocycle" or "carbocyclic residue" is intended to mean any stable 3- to 7-membered monocyclic or bicyclic or 7-to 13-membered bicyclic or tricyclic, any of which may be saturated, partially unsaturated, or aromatic. Examples of such carbocycles
20   include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane, [2.2.2]bicyclooctane, fluorenyl, phenyl, naphthyl,
25   indanyl, adamantyl, and tetrahydronaphthyl.

As used herein, the term "heterocycle" or "heterocyclic system" is intended to mean a stable 5-to 7-membered monocyclic or bicyclic or 7-to 10-membered bicyclic heterocyclic ring which is saturated partially
30   unsaturated or unsaturated (aromatic), and which consists of carbon atoms and from 1 to 4 heteroatoms independently selected from the group consisting of N, O and S and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a
35   benzene ring.   The nitrogen and sulfur heteroatoms may

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optionally be oxidized. The heterocyclic ring may be
attached to its pendant group at any heteroatom or
carbon atom which results in a stable structure. The
heterocyclic rings described herein may be substituted
5   on carbon or on a nitrogen atom if the resulting
compound is stable. A nitrogen in the heterocycle may
optionally be quaternized. It is preferred that when
the total number of S and O atoms in the heterocycle
exceeds 1, then these heteroatoms are not adjacent to
10   one another. It is preferred that the total number of S
and O atoms in the heterocycle is not more than 1. As
used herein, the term "aromatic heterocyclic system" or
"heteroaryl" is intended to mean a stable 5-to 7-
membered monocyclic or bicyclic or 7-to 10-membered
15   bicyclic heterocyclic aromatic ring which consists of
carbon atoms and from 1 to 4 heterotams independently
selected from the group consisting of N, O and S. It is
preferred that the total number of S and O atoms in the
aromatic heterocycle is not more than 1.

20   Examples of heterocycles include, but are not
limited to, acridinyl, azocinyl, benzimidazolyl,
benzofuranyl, benzothiofuranyl, benzothiophenyl,
benzoxazolyl, benzthiazolyl, benztriazolyl,
benztetrazolyl, benzisoxazolyl, benzisothiazolyl,
25   benzimidazolinyl, carbazolyl, 4aH-carbazolyl,
carbolinyl, chromanyl, chromenyl, cinnolinyl,
decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl,
dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl,
imidazolidinyl, imidazolinyl, imidazolyl, 1H-indazolyl,
30   indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl,
isobenzofuranyl, isochromanyl, isoindazolyl,
isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl,
isoxazolyl, methylenedioxyphenyl, morpholinyl,
naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl,
35   1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl,

1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl,
pyrimidinyl, phenanthridinyl, phenanthrolinyl,
phenazinyl, phenothiazinyl, phenoxathiinyl,
phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl,
5   pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl,
pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole,
pyridoimidazole, pyridothiazole, pyridinyl, pyridyl,
pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl,
pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizinyl,
10 quinoxalinyl, quinuclidinyl, tetrahydrofuranyl,
tetrahydroisoquinolinyl, tetrahydroquinolinyl, 6H-1,2,5-
thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl,
1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl,
thiazolyl, thienyl, thienothiazolyl, thienooxazolyl,
15 thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-
triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-
triazolyl, and xanthenyl. Preferred heterocycles
include, but are not limited to, pyridinyl, furanyl,
thienyl, pyrrolyl, pyrazolyl, pyrrolidinyl, imidazolyl,
20 indolyl, benzimidazolyl, 1H-indazolyl, oxazolidinyl,
benzotriazolyl, benzisoxazolyl, oxindolyl,
benzoxazolinyl, and isatinoyl. Also included are fused
ring and spiro compounds containing, for example, the
above heterocycles.

25   The term "amino acid" as used herein means an
organic compound containing both a basic amino group and
an acidic carboxyl group. Included within this term are
natural amino acids (e.g., L-amino acids), modified and
unusual amino acids (e.g., D-amino acids), as well as
30 amino acids which are known to occur biologically in
free or combined form but usually do not occur in
proteins. Included within this term are modified and
unusual amino acids, such as those disclosed in, for
example, Roberts and Vellaccio (1983) The Peptides, 5:
35 342-429, the teaching of which is hereby incorporated by

reference.   Natural protein occurring amino acids
include, but are not limited to, alanine, arginine,
asparagine, aspartic acid, cysteine, glutamic acid,
glutamine, glycine, histidine, isoleucine, leucine,
5   lysine, methionine, phenylalanine, serine, threonine,
tyrosine, tyrosine, tryptophan, proline, and valine.
Natural non-protein amino acids include, but are not
limited to arginosuccinic acid, citrulline, cysteine
sulfinic acid, 3,4-dihydroxyphenylalanine, homocysteine,
10   homoserine, ornithine, 3-monoiodotyrosine,
3,5-diiodotryosine, 3,5,5'-triiodothyronine, and
3,3',5,5'-tetraiodothyronine.   Modified or unusual amino
acids which can be used to practice the invention
include, but are not limited to, D-amino acids,
15   hydroxylysine, 4-hydroxyproline, an N-Cbz-protected
amino acid, 2,4-diaminobutyric acid, homoarginine,
norleucine, N-methylaminobutyric acid, naphthylalanine,
phenylglycine, ß-phenylproline, tert-leucine,
4-aminocyclohexylalanine, N-methyl-norleucine,
20   3,4-dehydroproline, N,N-dimethylaminoglycine,
N-methylaminoglycine, 4-aminopiperidine-4-carboxylic
acid, 6-aminocaproic acid,
trans-4-(aminomethyl)cyclohexanecarboxylic acid, 2-, 3-,
and 4-(aminomethyl)benzoic acid,
25   1-aminocyclopentanecarboxylic acid,
1-aminocyclopropanecarboxylic acid, and
2-benzyl-5-aminopentanoic acid.

The term "peptide" as used herein means a linear
compound that consists of two or more amino acids (as
30   defined herein) that are linked by means of a peptide
bond.   A "peptide" as used in the presently claimed
invention is intended to refer to a moiety with a
molecular weight of less than 10,000 Daltons, preferable
less than 5,000 Daltons, and more preferably less than
35   2,500 Daltons.   The term "peptide" also includes

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compounds containing both peptide and non-peptide components, such as pseudopeptide or peptidomimetic residues or other non-amino acid components. Such a compound containing both peptide and non-peptide

5   components may also be referred to as a "peptide analog".

A "pseudopeptide" or "peptidomimetic" is a compound which mimics the structure of an amino acid residue or a peptide, for example, by using linking groups other than

10   amide linkages between the peptide mimetic and an amino acid residue (pseudopeptide bonds) and/or by using non-amino acid substituents and/or a modified amino acid residue. A "pseudopeptide residue" means that portion of an pseudopeptide or peptidomimetic that is present in

15   a peptide.

The term "peptide bond" means a covalent amide linkage formed by loss of a molecule of water between the carboxyl group of one amino acid and the amino group of a second amino acid.

20   The term "pseudopeptide bonds" includes peptide bond isosteres which may be used in place of or as substitutes for the normal amide linkage. These substitute or amide "equivalent" linkages are formed from combinations of atoms not normally found in

25   peptides or proteins which mimic the spatial requirements of the amide bond and which should stabilize the molecule to enzymatic degradation.

The term "non-peptide" refers to a compound in comprised of preferably less than three amide bonds in

30   the backbone core compound or preferably less than three amino acids or amino acid mimetics.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the

scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate

5   with a reasonable benefit/risk ratio.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable

10   salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; and alkali or organic salts of acidic residues such as carboxylic acids. The pharmaceutically acceptable salts include the conventional non-toxic salts or the

15   quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric;

20   and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric,

25   toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, and isethionic.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by

30   conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally,

35   nonaqueous media like ether, ethyl acetate, ethanol,

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isopropanol, or acetonitrile are preferred. Lists of
suitable salts are found in *Remington's Pharmaceutical
Sciences*, 17th ed., Mack Publishing Company, Easton, PA,
1985, p. 1418, the disclosure of which is hereby
5   incorporated by reference.

"Stable compound" and "stable structure" are meant
to indicate a compound that is sufficiently robust to
survive isolation to a useful degree of purity from a
reaction mixture, and formulation into an efficacious
10 therapeutic agent.

Lyophilization aids useful in the preparation of
diagnostic kits useful for the preparation of
radiopharmaceuticals include but are not limited to
mannitol, lactose, sorbitol, dextran, Ficoll, and
15 polyvinylpyrrolidine (PVP).

Solubilization aids useful in the preparation of
radiopharmaceuticals and in diagnostic kits useful for
the preparation of said radiopharmaceuticals include but
are not limited to ethanol, glycerin, polyethylene
20 glycol, propylene glycol, polyoxyethylene sorbitan
monooleate, sorbitan monoloeate, polysorbates,
poly(oxyethylene)poly(oxypropylene)poly(oxyethylene)
block copolymers (Pluronics) and lecithin. Preferred
solubilizing aids are polyethylene glycol, and
25 Pluronics.

Bacteriostats useful in the preparation of
radiopharmaceuticals and in diagnostic kits useful for
the preparation of said radiopharmaceuticals include but
are not limited to benzyl alcohol, benzalkonium
30 chloride, chlorbutanol, and methyl, propyl or butyl
paraben.

EXPERIMENTAL

The integrity of a radiopharmaceutical is measured
by the radiochemical purity (RCP) of the radiolabeled
5   compound using ITLC or more preferably HPLC. The
advantage of using HPLC is that radio-impurities caused
by radiolytic degradation can be separated from the
radiopharmaceutical under optimized chromatographic
conditions. Improved stability over time for
10   radiopharmaceutical compositions of this invention can
be demonstrated by determining the change in RCP of the
radiolabeled compound in samples taken at representative
time points. The radiopharmaceutical compositions of
this invention are effective in maintaining the long-
15   term stability of samples that have been frozen, thawed,
and re-tested periodically for 5 days.

The initial RCP of a radiopharmaceutical is largely
dependent on radiolabeling conditions such as pH,
heating temperature and time. Once a
20   radiopharmaceutical is prepared in high yield, the
stability of the radiopharmaceutical composition is
measured by the RCP change of the radiopharmaceutical
over a certain period of time.

Materials. Acetic acid (ultra-pure), ammonium
25   hydroxide (ultra-pure), ascorbic acid (sodium salt), and
sodium gentisate were purchased from either Aldrich or
Sigma Chemical Co., and were used as received. $^{90}YCl_3$
and $^{111}InCl_3$ (in 0.05 N HCl) were purchased from NEN®, N.
Billerica, MA. High specific activity $^{177}LuCl_3$ was
30   obtained from University of Missouri Research Reactor,
Columbia, MO.

Analytical Methods. HPLC method 1 used a HP-1100
HPLC system with a UV/visible detector (λ = 220 nm), an
IN-US radio-detector, and a Zorbax C_{18} column (4.6 mm x

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250 mm, 80 Å pore size). The flow rate was 1 mL/min
with the mobile phase starting with 92% solvent A (0.025
M ammonium acetate buffer, pH 6.8) and 8% solvent B
(acetonitrile) to 90% solvent A and 8% solvent B at 18
5   min, followed by an isocratic wash using 40% of solvent
A and 60% solvent B from 19 to 25 min.

HPLC method 2 used a HP-1100 HPLC system with a
UV/visible detector (λ = 220 nm), an IN-US radio-
detector, and a Zorbax C_{18} column (4.6 mm x 250 mm, 80 Å
10   pore size). The flow rate was 1 mL/min with the mobile
phase starting with 92% solvent A (0.025 M ammonium
acetate buffer, pH 6.8) and 8% solvent B (acetonitrile)
to 80% solvent A and 20% solvent B at 18 min, followed
by an isocratic wash using 40% of solvent A and 60%
15   solvent B from 19 to 25 min.

HPLC method 3 used a HP-1100 HPLC system with a
UV/visible detector (λ = 220 nm), an IN-US radio-
detector, and a Zorbax C_{18} column (4.6 mm x 250 mm, 80 Å
pore size). The flow rate was 1 mL/min with an
20   isocratic mobile phase with 92% solvent A (0.025 M
ammonium acetate buffer, pH 6.8) and 8% solvent B
(acetonitrile) over 25 min, followed by an isocratic
wash using 40% of solvent A and 60% solvent B from 26 to
30 min.

25   The ITLC method used reverse phase C_{18} TLC plates
and a mixture of methanol, acetone and saline (2:1:1 =
v:v:v) as eluant. By this method, the radiolabeled
compounds migrate to the solvent front while
[^{90}Y]/[^{177}Lu]colloid and [^{90}Y]/[^{177}Lu]acetate remain at the
30   origin.

EXAMPLE 1

Preparation ^{90}Y-(2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-
[3-sulfo-2-(3-sulfo-2-{2-[1,4,7,10-tetraaza-4,7,10-

5   tris(carboxymethyl) cyclododecyl]acetylamino}propyl)-

propyl]ethyl}carbamoyl)propoxy]phenyl}sulfonyl)amino]-3-
({7-[(imidazol-2-ylamino)methyl]-1-methyl-4-oxo(3-
hydroquinolyl)}carbonylamino)propanoic Acid
Trifluoroacetate Salt (20 mCi) Using Ascorbic Acid (AA,

10   0.1 M or 20 mg/mL, pH = 7.35) as a buffer agent,
Transfer Ligand and Radiolytic Stabilizer.

(2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-
sulfo-2-{2-[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-

15   propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate salt was
prepared as disclosed in US Patent Application No.
09/456,300 and was subsequently dissolved in 0.1 M

20   ascorbic acid buffer (pH 7.35) to give a concentration
of 100 µg/mL. The resulting solution was immediately
degassed under vacuum for another 1 - 2 min. To a clean
sealed 5 mL vial was added 1.0 mL of 0.1 M ascorbic acid
(sodium salt) buffer (pH 7.35) containing 100 µg of

25   (2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-
{2-[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-

30   carbonylamino)propanoic acid trifluoroacetate. The
solution was degassed again under vacuum. Upon addition
of ~10 µL of ^{90}YCl$_3$ solution (20.5 mCi) in 0.05 N HCl,
the reaction mixture was heated at 95 °C for 5 min.
After cooling to room temperature, a sample of the

resulting solution was diluted 50-fold with saline
containing sodium gentisate (10 mg/mL), and was then
analyzed by HPLC (Method 1, injection volume = 5 μL).
The RCP was 99.3%. The retention time was 14.7 min.
5   The TLC (reverse phase C_{18} TLC) showed minimal (0.38%)
[^{90}Y]colloid and [^{90}Y]acetate impurities.

This clearly shows that ^{90}Y-(2S)-2-[({2,6-Dimethyl-
4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-[1,4,7,10-tetraaza-
4,7,10-tris(carboxymethyl)
10   cyclododecyl]acetylamino}propyl)-
-propyl]ethyl}carbamoyl)propoxy]phenyl}sulfonyl)amino]-
3-({7-[(imidazol-2-ylamino)methyl]-1-methyl-4-oxo(3-
hydroquinolyl)}carbonylamino)propanoic acid
trifluoroacetate can be prepared in high yield and
15   radiochemical purity using ascorbic acid as a buffer
agent for pH control and a weak transfer ligand to
prevent the formation of [^{90}Y]colloid. Based on the
results, a radiolabeling experiment was designed to find
optimal radiolabeling conditions in using ascorbic acid
20   as a buffering agent for pH control, a transfer ligand
to prevent [^{90}Y]colloid formation, and as a stabilizer
for the solution stability of ^{90}Y-(2S)-2-[({2,6-
Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-[1,4,7,10-
tetraaza-4,7,10-tris(carboxymethyl)
25   cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate. Four
factors were considered in the experimental design.
30   These include pH value (5, 6, and 7), heating time (5
min and 35 min), sodium ascorbic level (20 mg and 100
mg), and temperature (50 °C and 95 °C). Each condition
contains two vials. The activity level for each vial
was ~10 mCi. The reaction mixture from each vial was
35   characterized by HPLC and reverse phase C_{18} TLC.

Based on the radiolabeling results, it is clear
that (1) AA level does not have a significant effect on
the RCP as long as the heating temperature is 95 °C; (2)
the pH shows little effect on the RCP at pH = 5 - 7; (3)
5   a longer heating time gives slightly better RCP at 95
°C; and (4) heating temperature is the most dominant
factor on RCP.

EXAMPLE 2

10   **Preparation and Solution Stability of ^{90}Y-(2S)-2-**
[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-
[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}-

-propyl)propyl]ethyl}carbamoyl)-
15   **propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-**
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate (100 mCi
Level) Using Ascorbic Acid (AA, 20 mg/mL or 0.1 M, pH =
5.0) as the buffer agent, Transfer Ligand and Radiolytic
20   **Stabilizer.**

(2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-
{2-[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
25   ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate was
prepared as disclosed in US Patent Application No.
09/456,300 and was subsequently dissolved in 0.1 M AA
buffer (pH 5.0) to give a concentration of 100 µg/mL.
30   The resulting solution was immediately degassed under
vacuum for another 1 - 2 min. To a clean sealed 5 mL
vial was added 5.0 mL of 0.1 M ascorbic acid buffer (pH
5.0) containing 500 µg of (2S)-2-[({2,6-Dimethyl-4-[3-
(N-{2-[3-sulfo-2-(3-sulfo-2-{2-[1,4,7,10-tetraaza-

4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}propyl)

propyl]ethyl}carbamoyl)propoxy]phenyl}sulfonyl)amino]-3-
({7-[(imidazol-2-ylamino)methyl]-1-methyl-4-oxo(3-

5   hydroquinolyl)}carbonylamino)propanoic acid
trifluoroacetate. The solution was degassed again under
vacuum. Upon addition of ~75 µL of ^{90}YCl$_3$ solution
(101.5 mCi) in 0.05 N HCl, the reaction mixture was
heated at 95 °C for 30 min. After cooling to room

10   temperature, a sample of the resulting solution was
diluted 50-fold with saline containing sodium gentisate
(10 mg/mL), and was then analyzed by HPLC (Method 1,
injection volume = 5 µL). The resulting mixture was
then kept in a dry-ice box (-78 °C) for 5 days. Samples

15   were analyzed at t = 0 (RCP = 98.5%), 24 h (RCP =
98.4%), 68 h (RCP = 98.0%), and 120 h (RCP = 98.8%). The
retention time was 14.8 min.

This experiment clearly demonstrated that ^{90}Y-(2S)-2-
[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-

20   [1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}-

-propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-

25   carbonylamino)propanoic acid trifluoroacetate can be
readily prepared in high RCP (>98%) under the following
conditions: 500 µg (2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-[3-
sulfo-2-(3-sulfo-2-{2-[1,4,7,10-tetraaza-4,7,10-
tris(carboxymethyl)

30   cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate for 100
mCi of ^{90}Y in 5 mL of AA solution containing 100 mg AA,

35   pH=5.0, heating at 95 °C for 30 min and remains stable

for at least 5 days (RCP > 96%). Ascorbic acid can be
used as a buffer agent, a transfer ligand, and a
radiolytic stabilizer for the routine preparation and
stabilization of ^{90}Y-labeled biomolecules.

5

EXAMPLE 3

Preparation and Solution Stability of ^{111}In-(2S)-2-
[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-
[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)

10   cyclododecyl]acetylamino}-

-propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate **(2.8 mCi)**

15   **Using Ascorbic Acid (AA, 20 mg/mL or 0.1 M) as the**
Buffer agent, Transfer Ligand, and Radiolytic
Stabilizer.

      (2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-
sulfo-2-{2-[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)

20   cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate was
prepared as disclosed in US Patent Application No.

25   09/456,300 and was subsequently dissolved in 0.1 M
ascorbic acid buffer (pH 6.0) to give a concentration of
100 μg/mL. The resulting solution was immediately
degassed under vacuum for another 1 - 2 min. To a clean
sealed 5 mL vial was added 2.0 mL of 0.1 M ascorbic acid

30   buffer (pH 6.0) containing 150 μg of (2S)-2-[({2,6-
Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-[1,4,7,10-
tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}

propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate. The

5   solution was degassed again under vacuum. Upon addition
of ~7 µL of ^{111}InCl$_3$ solution (2.8 mCi) in 0.05 N HCl,
the reaction mixture was heated at 100 °C for 5 min.
After cooling to room temperature, a sample of the
resulting solution was then analyzed by HPLC (Method 3,

10   injection volume = 10 µL). The resulting mixture was
then kept at room temperature for 24 hours. Samples
were analyzed at t = 0 (RCP = 98.2%) and 24 h (RCP =
97.6%). The retention time was 11.7 min.

This clearly demonstrated that ^{111}In-(2S)-2-[({2,6-

15   Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-sulfo-2-{2-[1,4,7,10-
tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}

propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-

20   ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate could be
prepared in high yield using ascorbic acid as a buffer
agent, a transfer ligand and a radiolytic stabilizer.
^{111}In-(2S)-2-[({2,6-Dimethyl-4-[3-(N-{2-[3-sulfo-2-(3-

25   sulfo-2-{2-[1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)
cyclododecyl]acetylamino}propyl)propyl]ethyl}carbamoyl)-
propoxy]phenyl}sulfonyl)amino]-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic acid trifluoroacetate remains

30   stable in solution for at least 24 hours. Ascorbic acid
can be used as a buffer agent, a transfer ligand, and a
radiolytic stabilizer for the routine preparation and
stabilization of ^{111}In-labeled biomolecules.

EXAMPLE 4

Preparation and Solution Stability of [177]Lu-

DOTA/(2S)-2-{[(4-{3-[N-(2-{2-[(4S)-4-(N-{1-[N-(2-{4-[4-
({[[(1S)-1-Carboxy-2-({7-[(imidazol-2-ylamino)methyl]-1-

5   methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)ethyl]amino}sulfonyl)-3,5-
dimethylphenoxy]butanoylamino}ethyl)carbamoyl]-2-
sulfoethyl}carbamoyl)-4-aminobutanoylamino]-3-
sulfopropyl}ethyl)carbamoyl]propoxy}-2,6-

10   dimethylphenyl)sulfonyl]amino}-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic Acid Conjugate
Bis(trifluoroacetate) **Using Ascorbic Acid (AA, 20 mg/mL
or 0.1 M) as the buffer agent, Transfer Ligand and**

15   **Radiolytic Stabilizer.**

       To a clean sealed 5 mL vial was added 2.0 mL of 0.1
M ascorbic acid buffer (pH 6.0) containing 137 µg of
DOTA/(2S)-2-{[(4-{3-[N-(2-{2-[(4S)-4-(N-{1-[N-(2-{4-[4-
({[[(1S)-1-Carboxy-2-({7-[(imidazol-2-ylamino)methyl]-1-

20   methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)ethyl]amino}sulfonyl)-3,5-
dimethylphenoxy]butanoylamino}ethyl)carbamoyl]-2-
sulfoethyl}carbamoyl)-4-aminobutanoylamino]-3-
sulfopropyl}ethyl)carbamoyl]propoxy}-2,6-

25   dimethylphenyl)sulfonyl]amino}-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic Acid Conjugate
Bis(trifluoroacetate) which was prepared as disclosed in
US Patent Application No. 09/456,300. The solution was

30   degassed again under vacuum. Upon addition of ~6 µL of
[177]LuCl$_3$ solution (~17 mCi) in 0.05 N HCl, the reaction
mixture was heated at 95 °C for 45 min. After cooling
to room temperature, a sample of the resulting solution
was analyzed by HPLC (Method 2, injection volume = 2 µL)

35   and reverse phase C$_{18}$ TLC. The radiochemical purity was

94.9% at 0 h and 95% at 24 h post-labeling. The TLC
showed minimal [^{177}Lu]colloid and [^{177}Lu]acetate
impurities at the origin (~1.2% by TLC).

5   It is clear that ^{177}Lu- DOTA/(2S)-2-{[(4-{3-[N-(2-
{2-[(4S)-4-(N-{1-[N-(2-{4-[4-({[(1S)-1-Carboxy-2-({7-
[(imidazol-2-ylamino)methyl]-1-methyl-4-oxo(3-
hydroquinolyl)}carbonylamino)ethyl]amino}sulfonyl)-3,5-
dimethylphenoxy]butanoylamino}ethyl)carbamoyl]-2-
sulfoethyl}carbamoyl)-4-aminobutanoylamino]-3-
10   sulfopropyl}ethyl)carbamoyl]propoxy}-2,6-
dimethylphenyl)sulfonyl]amino}-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
carbonylamino)propanoic Acid Conjugate
Bis(trifluoroacetate) could be prepared in high yield
15   using ascorbic acid as a buffer agent, a transfer ligand
and a radiolytic stabilizer. ^{177}Lu- DOTA/(2S)-2-{[(4-{3-
[N-(2-{2-[(4S)-4-(N-{1-[N-(2-{4-[4-({[(1S)-1-Carboxy-2-
({7-[(imidazol-2-ylamino)methyl]-1-methyl-4-oxo(3-
hydroquinolyl)}carbonylamino)ethyl]amino}sulfonyl)-3,5-
20   dimethylphenoxy]butanoylamino}ethyl)carbamoyl]-2-
sulfoethyl}carbamoyl)-4-aminobutanoylamino]-3-
sulfopropyl}ethyl)carbamoyl]propoxy}-2,6-
dimethylphenyl)sulfonyl]amino}-3-({7-[(imidazol-2-
ylamino)methyl]-1-methyl-4-oxo(3-hydroquinolyl)}-
25   carbonylamino)propanoic Acid Conjugate
Bis(trifluoroacetate) remains stable in solution for at
least 24 hours. Ascorbic acid can be used as a buffer
agent, a transfer ligand, and a radiolytic stabilizer
for the routine preparation and stabilization of ^{177}Lu-
30   labeled biomolecules.

<u>Claims</u>

What is claimed is:

5   1.   A radiopharmaceutical composition comprising a
      radiolabeled chelator-biomolecule conjugate of the
      formula M-Ch-Ln-(BM)$_m$; and an amount of a compound of
      formula (I):

(I)

10
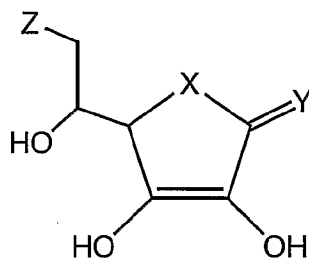
or a pharmaceutically acceptable salt thereof,

wherein

15   X is O, NR^1, or CHR^1;

      Y is O or S;

      Z is hydroxyl or halogen;

      R^1 is selected from: (C$_1$-C$_{10}$)alkyl substituted with
      0-5 R^2, (C$_3$-C$_{10}$)cycloalkyl substituted with 0-5 R^2, (C$_2$-
20   C$_{10}$)alkenyl substituted with 0-5 R^2, and aryl substituted
      with 0-5 R^2; and

R² is independently selected at each occurrence
from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and
SO_3H;

     wherein the amount of the compound of formula (I)

5 is effective to: (1) stabilize the radiolabeled
chelator-biomolecule conjugate of the formula M-Ch-Ln-
(BM)ₘ against radiation induced degradation and to at
least one of (2) control the pH of the
radiopharmaceutical composition and (3) prevent

10 radiometal colloid formation.

2.   The radiopharmaceutical composition of claim 1
wherein the amount is effective to: (1) stabilize the
radiopharmaceutical against radiation induced

15 degradation and (2) control the pH of the
radiopharmaceutical.

3.   The radiopharmaceutical composition of claim 1
wherein the amount is effective to: (1) stabilize the

20 radiopharmaceutical against radiation induced
degradation and (2 prevent radiometal colloid formation.

4.   The radiopharmaceutical composition of claim 1
wherein the amount is effective to: (1) stabilize the

25 radiopharmaceutical against radiation induced
degradation, (2) control the pH of the
radiopharmaceutical and (3) prevent radiometal colloid
formation.

5.   The radiopharmaceutical composition of claim 1 wherein X is O.

6.   The radiopharmaceutical composition of claim 1
5   wherein Y is O.

7.   The radiopharmaceutical composition of claim 1 wherein Z is hydroxyl.

10   8.   The radiopharmaceutical composition of claim 1 wherein m is 1 to about 5.

9.   The radiopharmaceutical composition of claim 1 wherein m is 1 or 2.

15

10.   The radiopharmaceutical composition of claim 1 wherein m is 1.

11.   The radiopharmaceutical composition of claim 1
20   wherein m is 1 to about 5; X is O; and Y is O.

12.   The radiopharmaceutical composition of claim 1 wherein m is 1 or 2; X is O; Y is O; and Z is hydroxyl.

25   13.   The radiopharmaceutical composition of claim 1 wherein m is 1; X is O; Y is O; and Z is hydroxyl.

14. The radiopharmaceutical composition of claim 1 wherein the concentration of the compound of formula (I) is about 2 mg/mL to about 200 mg/mL.

5   15. The radiopharmaceutical composition of claim 1 wherein the metallic radioisotope is present at a level of about 10 mCi to about 2000 mCi.

16. The radiopharmaceutical composition of claim 1
10   wherein the metallic radioisotope is present at a concentration of greater than about 5 mCi/mL.

17. The radiopharmaceutical composition of claim 1 wherein the radiolabeled chelator-biomolecule conjugate
15   of the formula $M-Ch-Ln-(BM)_m$ is a diagnostic radiopharmaceutical.

18. The radiopharmaceutical composition of claim 1 wherein the radiolabeled chelator-biomolecule conjugate
20   of the formula $M-Ch-Ln-(BM)_m$ is a therapeutic radiopharmaceutical.

19. The radiopharmaceutical composition of claim 1 wherein the biomolecule is an antibody.

25

20. The radiopharmaceutical composition of claim 1 wherein the biomolecule is an antibody fragment.

21.   The radiopharmaceutical composition of claim 1
wherein the biomolecule is a peptide.

22.   The radiopharmaceutical composition of claim 1
5   wherein the biomolecule is a peptidomimetic.

23.   The radiopharmaceutical composition of claim 1
wherein the biomolecule is a non-peptide.

10   24.   The radiopharmaceutical composition of claim 1
wherein the biomolecule is a cyclic IIb/IIIa receptor
antagonist; an RGD containing peptide; a fibrinogen
receptor antagonist; a IIb/IIIa receptor ligand; a
ligand for the polymerization site of fibrin; a laminin
15   derivative; a ligand for fibrinogen; a thrombin ligand;
an oligopeptide that corresponds to the IIIa protein; a
hirudin-based peptide; a IIb/IIIa receptor ligand; a
thrombus, platelet binding, or atherosclerotic plaque
binding peptide; a fibrin binding peptide; a hirudin-
20   based peptide; a fibrin binding protein; a guanine
derivative that binds to the IIb/IIIa receptor; a
tyrosine derivative; a leukocyte binding peptide; a
chemotactic peptide; a leukostimulatory agent; an LTB4
antagonist; a somatostatin analog; a selectin binding
25   peptide; a biological-function domain; a platelet factor
4 or growth factor; a compound that binds to a receptor
that is expressed or upregulated in angiogenic tumor
vasculature; a peptide, polypeptide or peptidomimetic
that binds with high affinity to the receptors VEGF
30   receptors Flk-1/KDR, Flt-1, or neuropilin-1; a peptide,
polypeptide or peptidomimetic that binds to $\alpha v \beta 3$, $\alpha v \beta 5$,

α5β1, α4β1, α1β1, or α2β2; a compound that interacts
with receptor tyrosine kinases; a protein, antibody,
antibody fragment, peptide, polypeptide, or
peptidomimetic that binds to receptors or binding sites
5   on a tissue, organ, enzyme or fluid; a ß-amyloid protein
that has been demonstrated to accumulate in patients
with Alzheimer's disease; an atrial naturetic factor
derived peptide that binds to myocardial or renal
receptor; an antimyosin antibody that binds to areas of
10   infarcted tissue; or a nitroimidazole derivative that
localizes in hypoxic areas in vivo.

25.   The radiopharmaceutical composition of claim 1
wherein the chelator is a cyclic or acyclic
15   polyaminocarboxylate, a diaminedithiol, a
triamidemonothiol, a monoaminemonoamidedithiol, a
monoaminediamidemonothiol, a diaminedioxime, or a
hydrazine.

20   26.   The radiopharmaceutical composition of claim 1
wherein the chelator is tetradentate, with donor atoms
selected from nitrogen, oxygen and sulfur.

27.   The radiopharmaceutical composition of claim 1
25   wherein the chelator is diethylenetriaminepentaacetic
acid (DTPA); 1,4,7,10-tetraazazcyclododecane-1,4,7,10-
tetraacetic acid (DOTA); 1,4,8,11-
tetraazazcyclotetradecane-1,4,8,11-tetraacetic acid
(TETA); 1,4,7,10-tetraazazcyclododecane-1,4,7-triacetic
30   acid (DO3A); 2-Benzyl-1,4,7,10-tetraazazcyclododecane-

1,4,7,10-tetraacetic acid (2-Bz-DOTA); alpha-(2-
phenethyl)-1,4,7,10-tetraazacyclododecane-1-acetic-
4,7,10-tris(methylacetic) acid; 2-benzyl-
cyclohexyldiethylenetriaminepentaacetic acid; 2-benzyl-
6-methyl-diethylenetriaminepentaacetic acid; or 6,6"-
bis[N,N,N",N"-tetra(carboxymethyl)aminomethyl]-4'-(3-
amino-4-methoxyphenyl)-2,2':6',2"-terpyridine.

28. The radiopharmaceutical composition of claim 1
wherein the metallic radioisotope is ^{177}Lu, ^{149}Pm, ^{153}Sm,
^{166}Ho, ^{90}Y, ^{111}In, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{99m}Tc, ^{117m}Sn, ^{203}Pb, ^{177}Lu,
^{47}Sc, ^{109}Pd, ^{105}Rh, ^{186}Re, ^{188}Re, ^{60}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{97}Ru,
or ^{212}Bi.

29. The radiopharmaceutical composition of claim 1
wherein the metallic radioisotope is ^{99m}Tc, ^{117m}Sn, ^{111}In,
^{203}Pb, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{177}Lu, ^{149}Pm, ^{153}Sm, ^{166}Ho, ^{47}Sc,
^{109}Pd, ^{105}Rh, ^{186}Re, ^{188}Re, ^{60}Cu, ^{62}Cu, ^{64}Cu or ^{67}Cu.

30. The radiopharmaceutical composition of claim 1
wherein the metallic radioisotope is ^{111}In, ^{90}Y, or ^{177}Lu.

31. A radiopharmaceutical composition comprising a
radiolabeled chelator-biomolecule conjugate of the
formula M-Ch-Ln-(BM)$_m$; and a compound of formula (I):

(I)
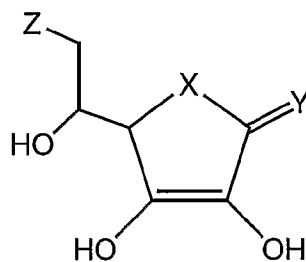
or a pharmaceutically acceptable salt thereof;

5   wherein

M is a metallic radioisotope;

Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule;

10   m is 1 to about 10;

X is O, NR^1, or CHR^1;

Y is O or S;

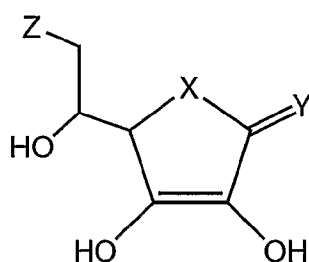Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with

15   0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2;

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and

20   SO_3H;

provided the radiopharmaceutical composition does not comprise an additional buffering agent or an additional chelating agent.

5 32. A method for buffering a radiopharmaceutical comprising contacting the radiopharmaceutical with an amount of a compound of formula (I):

10   (I)

or a pharmaceutically acceptable salt thereof, wherein

        X is O, NR^1, or CHR^1;

15   Y is O or S;

        Z is hydroxyl or halogen;

        R^1 is selected from: (C_1-C_{10})alkyl substituted with 0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted

20 with 0-5 R^2;

        R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and SO_3H;

wherein the amount is effective to control the pH
of the radiopharmaceutical.

33. The method of claim 32 wherein the
5 radiopharmaceutical is a radiolabeled chelator-
biomolecule conjugate of the formula M-Ch-Ln-(BM)$_m$;
wherein

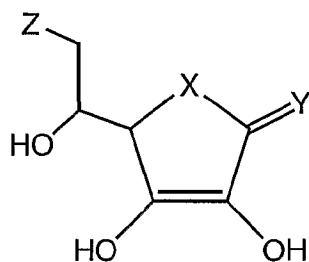M is a metallic radioisotope;

Ch is a metal chelator;

10 Ln is an optional linking group;

BM is a biomolecule; and

m is 1 to about 10.

34. The method of claim 32 wherein the buffering agent
15 controls the pH of the radiopharmaceutical during at
least one of the preparation, release, storage, and
transportation of the radiopharmaceutical.

35. A method for chelating a radiopharmaceutical
20 comprising contacting the radiopharmaceutical with an
amount of a compound of formula (I):

$$\text{(structure with Z, X, Y, HO, HO, OH)}$$

(I)

or a pharmaceutically acceptable salt thereof,

5   wherein

   X is O, NR^1, or CHR^1;

   Y is O or S;

   Z is hydroxyl or halogen;

   R^1 is selected from: (C_1-C_{10})alkyl substituted with

10   0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2;

   R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and SO_3H;

15   wherein the amount is effective to prevent radiometal colloid formation.

36. The method of claim 35 wherein the radiopharmaceutical is a radiolabeled chelator-

20   biomolecule conjugate of the formula $M-Ch-Ln-(BM)_m$;

   wherein

   M is a metallic radioisotope;

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Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule; and

m is 1 to about 10.

5

37.   The method of claim 35 wherein the chelating agent
prevents radiometal colloid formation during at least
one of the preparation, release, storage, and
transportation of the radiopharmaceutical.

10

38.   A method for stabilizing a radiopharmaceutical
against radiation induced degradation and at least one
of (1) controlling the pH of the radiopharmaceutical and
(2) preventing radiometal colloid formation; comprising
15   contacting the radiopharmaceutical with an amount of a
compound of formula (I):
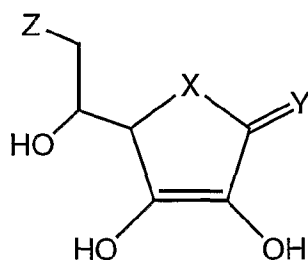
(I)

20

or a pharmaceutically acceptable salt thereof,

wherein

X is O, NR^1, or CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with
0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, $(C_2-$
5    $C_{10})$alkenyl substituted with 0-5 R^2, and aryl substituted
with 0-5 R^2;

R^2 is independently selected at each occurrence
from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and
SO_3H;

10   wherein the amount is effective to: (1) stabilize
the radiopharmaceutical against radiation induced
degradation and to at least one of (2) control the pH of
the radiopharmaceutical and (3) prevent radiometal
colloid formation.

15

39.   The method of claim 38 wherein the amount is
effective to: (1) stabilize the radiopharmaceutical
against radiation induced degradation and (2) control
the pH of the radiopharmaceutical.

20

40.   The method of claim 38 wherein the amount is
effective to: (1) stabilize the radiopharmaceutical
against radiation induced degradation and (2) prevent
radiometal colloid formation.

25

41.   The method of claim 38 wherein the amount is
effective to: (1) stabilize the radiopharmaceutical
against radiation induced degradation, (2) control the

pH of the radiopharmaceutical and (3) prevent radiometal colloid formation.

42. The method of claim 38 wherein the
5   radiopharmaceutical is a radiolabeled chelator-
biomolecule conjugate of the formula $M-Ch-Ln-(BM)_m$
wherein

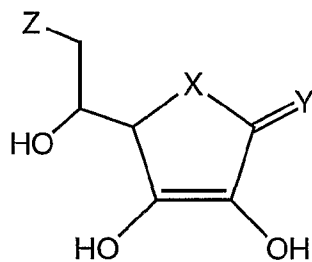M is a metallic radioisotope;

Ch is a metal chelator;

10   Ln is an optional linking group;

BM is a biomolecule; and

m is 1 to about 10.

43. The method of claim 38 wherein the amount is
15   effective to: stabilize the radiopharmaceutical against
radiation induced degradation and to at least one of (2)
control the pH of the radiopharmaceutical and (3)
prevent radiometal colloid formation; during at least
one of the preparation, release, storage, and
20   transportation of the radiopharmaceutical.

44. A method for preparing a stable radiopharmaceutical
composition comprising contacting a radiolabeled
chelator-biomolecule conjugate of the formula $M-Ch-Ln-$
25    $(BM)_m$; and an amount of a compound of formula (I):

(I)

or a pharmaceutically acceptable salt thereof,

5   wherein

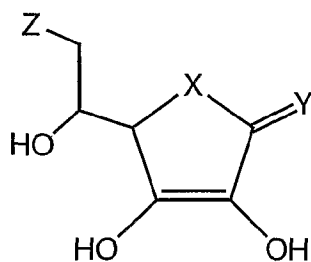X is O, NR^1, or CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with

10   0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2; and

R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and

15   SO_3H;

wherein the amount of the compound of formula (I) is effective to: (1) stabilize the radiolabeled chelator-biomolecule conjugate of the formula M-Ch-Ln-$(BM)_m$ against radiation induced degradation and to at

20   least one of (2) control the pH of the radiopharmaceutical composition and (3) prevent radiometal colloid formation.

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45.   A kit comprising a sealed vial comprising a
predetermined quantity of a radiolabeled chelator-
biomolecule conjugate of the formula M-Ch-Ln-(BM)$_m$, and
an amount of a compound of formula (I):

5

(I)

or a pharmaceutically acceptable salt thereof,

10   wherein

M is a metallic radioisotope;

Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule;

15   m is 1 to about 10;

X is selected from O, NR^1, and CHR^1;

Y is O or S;

Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with

20   0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted
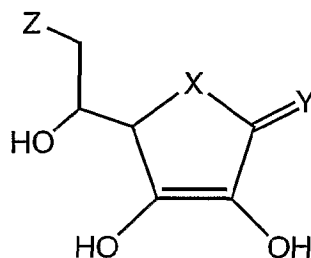with 0-5 R^2; and

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R^2 is independently selected at each occurrence from: NH$_2$, OH, CO$_2$H, C(=O)NH$_2$, NHC(=NH)NH$_2$, PO$_3$H$_2$, and SO$_3$H;

wherein the amount is effective to: (1) stabilize the radiolabeled chelator-biomolecule conjugate of the formula M-Ch-Ln-(BM)$_m$ against radiation induced degradation and to at least one of (2) control the pH of the radiopharmaceutical and (3) prevent radiometal colloid formation.

46. A kit comprising (a) a first vial comprising a predetermined quantity of a radiolabeled chelator-biomolecule conjugate of the formula M-Ch-Ln-(BM)$_m$; and an amount of a compound of formula (I):

(I)

or a pharmaceutically acceptable salt thereof,

wherein

M is a metallic radioisotope;

Ch is a metal chelator;

Ln is an optional linking group;

BM is a biomolecule;

m is 1 to about 10;

X is selected from O, NR^1, and CHR^1;

Y is O or S;

5   Z is hydroxyl or halogen;

R^1 is selected from: (C_1-C_{10})alkyl substituted with 0-5 R^2, (C_3-C_{10})cycloalkyl substituted with 0-5 R^2, (C_2-C_{10})alkenyl substituted with 0-5 R^2, and aryl substituted with 0-5 R^2; and

10   R^2 is independently selected at each occurrence from: NH_2, OH, CO_2H, $C(=O)NH_2$, $NHC(=NH)NH_2$, PO_3H_2, and SO_3H;

wherein the amount is effective to: (1) stabilize the radiopharmaceutical against radiation induced
15   degradation and to at least one of (2) control the pH of the radiopharmaceutical and (3) prevent radiometal colloid formation; and

(b)   a second vial comprising a pharmaceutically acceptable carrier or diluent.